The Journal of Laboratory and Clinical Medicine

VOL. XVIII ST. LOUIS, MO., JANUARY, 1933 NO. 4

CLINICAL AND EXPERIMENTAL

PATHOGENIC YEASTS AND YEAST-LIKE ORGANISMS

REPORT OF A CASE IN MINNESOTA SIMULATING COCCIDIOIDAL GRANULOMA*

DONALD C. BEAVER, M.D., AND EMIL D. FURRER, M.D., ROCHESTER, MINN.

YEASTS AND YEAST-LIKE FUNGI

MANY varieties of unicellular fungi called yeasts and similar forms producing mycelium, exist in nature. We are directly concerned with these fungi in this paper, since some of them possess pathogenic properties and may constitute the exciting agents of granulomatous and purulent inflammations of man.

True yeasts are defined by mycologists as unicellular fungi, spherical or oval, reproducing by budding or fission, and by sexual sporulation (ascospores), and usually inducing alcoholic fermentation in solutions of sugar. Occasionally, under special conditions, rudimentary mycelium may be evolved.

Yeast-like, that is, unicellular budding forms, are not alone distinctive of true yeasts. They also occur in all of the great classes of fungi, as a temporary mode of growth, in response to environment. The unicellular growth may in fact temporarily supplant the mycelial form in fungi inherently capable of forming mycelium. When fungi assume such unicellular formations they are referred to as yeast-like, but the term as used in a restricted sense designates those fungi which exhibit the yeast-like growth as a common feature of their development.

We may presume, as Henrici did, that the yeasts have been derived from more complex fungi, representing those which have permanently lost the power to form true mycelium. Their relationship to the more complex mycelial fungi is shown by their occasional attempts to form rudimentary mycelium, by their similarity to the yeast-like cells of certain mycelial fungi, and by their methods of sexual sporulation, in all, affording rather certain knowledge of their ancestry. Thus, it has been possible to classify side by side the unicellular yeasts and mycelial-producing fungi,

*From The Mayo Clinic and The Mayo Foundation.
Received for publication, March 23, 1932.

the essential difference being that mycelium is formed in one and only single budding cells in the other.

The precise classification for true yeasts and the related mycelium-producing fungi is schematically shown (adapted from Castellani[4]) in Table I.

TABLE I

YEASTS AND ENDOMYCES

ENMYCETES (TRUE FUNGI)			
CLASS	ORDER	FAMILY	GENUS
Ascomycetes. Ascospores (endospores) present; mycelium septate when present	Saccharomycetales. Asci not gathered into definite perithecia	Saccharomycetaceae (Rees). No definite mycelium	Saccharomyces (Meyer). True yeasts. Unicellular budding forms; occasionally rudimentary mycelium; reproducing by smooth ascospores; producing alcoholic fermentation of sugar solutions
		Endomycetaceae (Rees). Mycelium present	Endomyces (Rees). Yeast-like and mycelium formations; reproducing by ascospores, external spores and spores situated inside the mycelial tubes

TABLE II

YEAST-LIKE ORGANISMS OF FUNGI IMPERFECTI

ENMYCETES (TRUE FUNGI)				
CLASS	ORDER	SUBORDER	FAMILY	GENUS
Fungi imperfecti. Ascospores absent; mycelium septate when present	Thallosporales. Reproduction by thallospores (thallus or body secondarily adapted to reproduction) mycelial hyphae more than 1 micron in diameter	Blastosporineae. Reproduction by blastospores (a budding thallospore)	Cryptococcaceae (Kützing). Hyphae and spores alike, that, is yeast-like	Torula (Persoon). Usually nonpathogenic and not producing alcoholic fermentation
				Cryptococcus (Kützing). Pathogenic, with well developed double contour
			Oosporaceae (Saccardo). Mycelial hyphae present; spores typically in chains	Oidium (Link). Hyphae, long branched, terminating in chains of spores; gas not formed in carbohydrates
				Monilia (Persoon). In situ budding forms and mycelium; in cultures, mostly budding; some filaments; dextrose fermented with gas

As the yeasts may be interpreted as having been derived from more complex fungi, so also are less complex types probably derived from them, and the closely related Endomyces. These more simple types are regarded as imperfect fungi but they usually possess a prototype among those fungi which have perfect classifications. The imperfection consists in an unknown sexual sporulating stage. The imperfect fungi, a more or less heterogenous group, many of which are yeast-like, have been classified together under the name of fungi imperfecti. In referring to them Henrici remarked that the imperfection of the organisms is not entirely the result of an imperfect cycle, but in part, at least, due to our inadequate knowledge

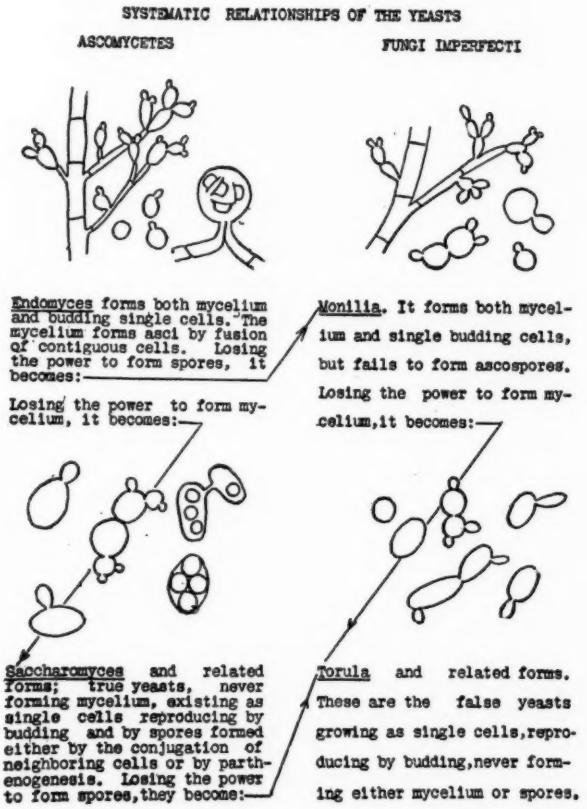

SYSTEMATIC RELATIONSHIPS OF THE YEASTS
ASCOMYCETES
FUNGI IMPERFECTI
Endomyces forms both mycelium and budding single cells. The mycelium forms asci by fusion of contiguous cells. Losing the power to form spores, it becomes:
Monilia. It forms both mycelium and single budding cells, but fails to form ascospores. Losing the power to form mycelium, it becomes:
Losing the power to form mycelium, it becomes:
Saccharomyces and related forms; true yeasts, never forming mycelium, existing as single cells reproducing by budding and by spores formed either by the conjugation of neighboring cells or by parthenogenesis. Losing the power to form spores, they become:
Torula and related forms. These are the false yeasts growing as single cells, reproducing by budding, never forming either mycelium or spores.

Fig. 1.—Systematic relationships of the yeasts.

of them. It is thus evident that their present classification is a means toward an end and not final.

Fungi imperfecti are of several orders, but the organisms pertinent to this consideration are classified in the order Thallosporales. A schematic illustration of their classification (adapted from Castellani[4]) is given in Table II.

Henrici schematically shows the relation which exists between Saccharomyces and Endomyces, Monilia and Torula. (Fig. 1.*)

According to Guilliermond and Tanner, yeasts were first described by Leewenhoek. Mitscherlick, Cagnard-Latour, Schwann and Kützing, about 1825, demon-

*Reprinted by permission from "Molds, Yeasts and Actinomycetes" by Arthur T. Henrici, published by John Wiley and Sons, Inc.

strated that yeasts of beer and wine were composed of cells which multiplied by budding. Endospores in yeasts were first observed by Schwann in 1839. He proved that they could be freed by rupturing the wall of the cell. Although yeasts had been closely allied with our knowledge of fermentation, their true significance dates from Pasteur's experiments in 1859. Hansen's description of their life cycle, their systematic relationship and classification, has been generally accepted. The discovery of zymase by Buchner, by which our knowledge of yeast nutrition and the mechanism of alcoholic fermentation has been considerably advanced, also has been fundamental.

Yeast-like fungi have been known since Link's description of Oidium lactis in 1809. Ricketts gave the characteristics of this genus apparently as defined by Link as follows: "A genus of fungi, having an elementary organization many species living as moulds. They have more or less ramifying and segmented filaments, which produce at their extremities short cells, very often rounded. These cells, which are united end to end, rosary-like, detach themselves when mature."

PATHOGENIC YEAST AND YEAST-LIKE ORGANISMS

About 1840, Robin and others, established Oidium albicans as the cause of thrush and other systemic lesions of man. Its relationship to human disease is well reviewed in Ricketts' monograph. The present tendency among mycologists is to regard the causative organisms of thrush as belonging to the genus Monilia; Castellani differentiated the two genera by gas fermentation reactions in dextrose: Monilia causes marked fermentation, Oidium little, if any; Henrici differentiated on the basis of forming free cells; in Oidium they arise by disarticulation of the mycelium, and in Monilia by budding from the mycelium. Castellani, however, believes that the clinical syndrome of thrush may result from a variety of infecting organisms, of the genera Oidium, Monilia, Endomyces, and Saccharomyces. The Oidium (Monilia), it appears, was the first of the yeasts or yeast-like organisms to be associated with disease of human beings.

The pathogenicity of yeasts and yeast-like forms for experimental animals, as demonstrated by Popoff, Rabinowitsch, Sanfelice,[32, 33, 34] Roncali, Weis and others, in 1872 and later, was the next advance in our knowledge that these fungi have pathogenic properties. Their description as the "parasites of cancer," the consideration of which occupied the attention of investigators[29, 30, 31, 32, 33, 34] in the latter part of the ninteenth century, is of historic significance only, since the idea has long been abandoned.[9, 22]

The association of true yeasts as the cause of disease of man is credited to Busse[2, 3] in 1894. In Busse's case, the patient was a woman, aged thirty-one years. Multiple, disseminate, superficial and deep subcutaneous abscesses developed secondary to a periosteal lesion of the tibia, which resembled a softened giant-cell sarcoma. At necropsy systemic abscesses were also found. In the original "tumor," in the pus from the abscesses, and in the blood, Busse found numerous, doubly contoured, rounded or ovoid bodies which were classified by cultural methods as yeasts in the genus Saccharomyces. He named the organism Saccharomyces hominis and the disease saccharomycosis hominis. Using material from this case, Buschke concurred in most essentials, independently, with Busse's observations.

Curtis[6, 7] in 1895 in France, published a second case of true yeast infection,

although there seems to be more doubt concerning the nature of the organism in this case. Curtis' patient presented a "myxomatous tumor" of the thigh. From the so-called tumor, organisms similar to those described by Busse were obtained, and although a cure had apparently been affected by surgical removal of the growth, Greenfield stated that this patient died ten months later of meningitis (possibly saccharomycetic).

There has been much controversy about these and similar cases of yeast infection, since there has been difficulty in accurately evaluating the original classifications. Stoddard and Cuttler and Greenfield classified the Busse-Buschke organism as Saccharomyces, while Castellani[4] and Vuillemin regarded it as Cryptococcus (a genus created by Vuillemin to accommodate the pathogenic Torula-false yeasts). Greenfield and Castellani classified the Curtis organism as Saccharomyces, and Stoddard and Cuttler left it unclassified. In any event, one must conclude that infections in man, due to true yeasts, are comparatively rare.

The stimulus to other investigators to establish yeasts as the parasites of malignant tumors was provided by Busse's suggestion, in his first report, that the tibial lesion which his patient exhibited resembled a softened giant-cell sarcoma, and by Curtis' report, which described a myxomatous tumor, both apparently having yeasts as etiologic factors.

In 1896, Gilchrist[10] reported a case of scrofuloderma on the back of a man's hand, in which large numbers of rounded, doubly contoured, refractive bodies, measuring 10 to 16 microns in diameter, with numerous budding forms present, were observed. The case antedated Busse's report by six months, but since cultural studies were not obtainable, the identity of this organism was not disclosed.

Gilchrist and Stokes, in 1896,[11] presented a second case of a purely cutaneous disease termed by them pseudolupus vulgaris, in the tissues of which organisms similar to those previously described by Gilchrist and corresponding to those of Busse, were revealed. In cultures the organism appeared as a mycelial growth with distal and lateral cells or conidia in place of budding yeast-like cells, as had been observed in the tissues. For this reason and also because alcoholic fermentation did not result when grown in solutions of sugar, the organism was classified as an Oidium. In supplementary studies,[12] however, due to uncertainty in the classification of the yeasts, Gilchrist and Stokes reversed their former opinion and described the organism as a yeast, naming the parasite Blastomyces dermatitidis and the disease blastomycosis (the term Blastomyces was at that time quite widely used for certain yeasts). It was found that a number of "wild yeasts" do not ferment solutions of sugar and do form mycelium.

Wernicke, from South America, in 1892, presented a case of doubtful mycosis fungoides in the lesions of which he observed protozoon-like forms. Rixford and Gilchrist in 1896 described similar organisms from two cases of a generalized chronic granulomatous disease, both of which had come from California. The parasites did not grow in artificial cultures so they concluded that they were dealing with a new form of protozoon infection resembling coccidiosis. Rixford and Gilchrist named the organisms Coccidioides immitis (or pyogenes), and the disease coccidioidal pseudotuberculosis. The pathologic anatomy of the lesions resembled closely that found in tuberculosis, fundamentally differing, however, in that pyogenic reactions were also frequent. The organisms were spherical, unicellular

bodies, varying from 7 to 27 microns in diameter and consisting in their encapsulated condition of a thick, doubly contoured capsule enclosing a finely granular protoplasm. They developed by sporulation and often as many as 100 small spores or "sporozoites" were set free by the bursting of the capsule. They were occasionally found to be intracellular, but as a rule they were extracellular.

In 1900, Ophüls and Moffitt succeeded in demonstrating the true nature of the so-called protozoon bodies. From a case, similar to those described by Wernicke, and Rixford and Gilchrist, cultures of the protozoon bodies revealed them to be the fructification stage of a mold. The mold developed in cultures as a mycelium with aerial hyphae, and with bodies resembling oidia. In 1905, Ophüls after further study classified the fungus as a member of the genus Oidium, and proposed the designation Odium coccidioides for the organism and oidiomycosis for the disease. He recognized the similarity between the coccidioidal organism and the organism of blastomycosis. He had been able to see the evolution of mycelium from the "protozoon-like" bodies when the latter were freed from the tissue in cultures. Wolbach in 1904 and MacNeal and Taylor in 1914 demonstrated its completed life cycle by showing that the mycelium, when introduced into susceptible animals, evolved the protozoon-like bodies.

The impression, generally held, that blastomycosis as described by Gilchrist and Stokes was only a cutaneous disease, was disproved by Walker and Montgomery in 1902 when they demonstrated a systemic disease, complicating blastomycosis of the skin, caused by organisms essentially identical with those that had been found in the cutaneous lesions.

In 1916, Stoddard and Cuttler reported a fourth type of infection due to yeast-like organisms. Their cases were peculiar in that the central nervous system was the principal site of involvement. The associated organisms were yeast-like in both the tissues and on cultures, evidencing growth by budding, without sporulating. Because of the yeast-like form which the organisms assumed under all conditions and due to failure to demonstrate reproduction by sporulation, they were designated as Torula, with the specific name Torula histolytica. The disease was called torulosis. The organisms, as they appeared in the tissues, had the apparently distinctive characteristic of occurring in zoogleal masses due to elaboration of a gelatinous matrix. The lesions appeared as either cystic or nodular areas in the brain or meninges, and were granulomatous in nature, sometimes with caseation necrosis. McGehee and Michelson have also recorded local infectious processes in other anatomic situations due to Torula.

Although many other types of yeasts and yeast-like pathogens have been described, space does not permit their consideration here. Neither does it permit a complete consideration of their history.

COMMON FEATURES OF THE YEAST AND YEAST-LIKE PARASITES AND THEIR INFECTION

In agreement with the literature we have classified four rather well-defined diseases, each having a specific yeast or yeast-like organism as its etiology. On the other hand, it is only necessary casually to review the reports to be convinced that there exist many common features of morphology and growth. The organisms all maintain doubly contoured yeast-like morphology in the tissues and except in the case of Coccidioides, growth by budding is characteristic. In cultures some main-

tain the distinctly yeast-like morphology, others produce mycelium, but common to all are the yeast-like or oidial formations. Each of the diseases is characterized by chronic granulomatous reactions, more or less closely resembling tuberculosis, and also eliciting a variable pyogenic reaction. Each may have local cutaneous or subcutaneous manifestations or result in systemic infection. The portals of entry in each are similar: skins, lungs or other (unknown) internal organs permit the invasion. Referring to the clinical syndromes, Montgomery stated that: "Coccidioidal granuloma, in spite of the literature to the contrary, may be indistinguishable clinically from certain cases of blastomycosis." The same statement could be broadened to include all of the diseases referred to in this paper. Any of the so-called distinctive clinical or pathologic characteristics of these diseases may be duplicated by others of the group. Castellani[4, 5] remarked that each of these diseases is often referred to as blastomycosis, again suggesting the likeness of one to another.

This intimates that these diseases and their organisms are more closely related than might be assumed by the varied classifications encountered in their descriptions; certainly more closely related to one another than sporotrichosis or actinomycosis is related to them. For example, they exhibit a relationship comparable to that which exists between the various diseases caused by the parasites of the order Actinomycetales.

Variations might be explained on the basis of adaptation to environment such as is shown by Blastomyces (Gilchrist). Ricketts, Henrici and others have shown that this parasite may assume three different types of growth in cultures, namely, yeast-like, oidium-like, and hyphomycetoid (Ricketts), or mealy, prickly and wooly (Henrici), and that these characteristics are influenced by environment. All the different stages, Henrici found, might occur in one and the same organism when subjected to changes which influence growth. In the tissues of the host some of the variations exhibited by the organisms could be similarly explained. The variations in the tissue reactions may be determined by the adaptive changes of the organisms or by other factors which influence the relationship between virulence of the organisms and resistance of the host.

Ricketts, in 1901, was the first to propose a simplified classification for the yeasts and yeast-like pathogenes and the diseases resulting from them. He studied material and cultures from seventeen cases of the various diseases caused by yeast and yeast-like organisms. Fourteen cases were representative of cutaneous blastomycosis, the remaining three were of the Busse-Buschke case, the Curtis case, and the Ophüls-Moffitt case. Ricketts proposed to include all in the genus Oidium, as defined by Link and to name the diseases oidiomycosis. His conclusions have not been generally accepted because of his insistence on the genus Oidium for all. In placing the organisms in a common genus and relating the diseases to variations of an identical process, he proceeded farther than either contemporary or subsequent investigators have been willing to follow. To quote his conclusions in part: "The so-called protozoic disease of Posadas, Wernicke and others; Busse's and Curtis' saccharomycosis hominis; and Gilchrist's blastomycetic dermatitis are various manifestations of the same disease. . . . The organisms isolated from the various cases differ in minor respects among themselves, but are so closely related morphologically as to justify their inclusion in a common genus, Oidium; they are

thus analogous in a pathogenic sense to the fungi which cause actinomycosis and to those causing trichophytosis.''

CLASSIFICATION OF THE PATHOGENIC YEASTS AND YEAST-LIKE ORGANISMS

The organisms of Busse's and Curtis' cases and a few others, correspond to true yeasts. Their proper designation should be Saccharomyces, as originally proposed for them.

Torula of Stoddard and Cuttler are also probably in the present stage of knowledge, properly designated as such, or as Cryptococcus. Characteristics which might serve better to classify them have not been described.

With Blastomyces the systematic position is not so clear. The organism is neither a true nor a false yeast, although it has certain yeast-like forms. Ricketts and Stoddard and Cuttler allocated it to the genus Oidium. Henrici also favored this view, using the designation Oidium for those forms which give rise to free cells by disarticulation of the mycelium as in Oidium lactis.

Coccidioides, because of its apparent reproduction by endosporulation in the tissues and because of its cultural characteristics of mycelial growth, has been generally assigned to the Ascomycetes (family Endomycetaceae). Although its cultural characteristics support Ophüls' view that it is an Oidium, endogenous sporulation as it occurs in the tissues is not consistent with the genus Oidium, or other types of Fungi imperfecti. Endospores in such numbers, however, are not found in other species of the Endomycetaceae, the family to which this parasite has been provisionally assigned in present classifications.[14] Also opposed to the classification of Coccidioides as an Ascomycete is the fact that no trace of sexuality has been found in the development of the spores (Henrici).

These individual designations are presented with the understanding that the organisms usually offer sufficiently distinctive characteristics to classify as such, but also with the knowledge that they constitute a biologically unsettled group subject to mutations and variations among themselves, thus exhibiting varied growth potentials, each organism being fundamentally closely related as would be the case if each were derived from a common form. In place of allocating all of the organisms to the genus Oidium and designating all of the diseases as oidiomycosis, as Ricketts did to satisfy this relationship, it seems preferable to refer to them according to their predominating characteristics, using specific names for this predominance as we have done, but with an appreciation of their close relationships. We may illustrate this point of close relationship by referring back to the schema, as proposed by Henrici (Fig. 1), substituting these pathogenic forms for the homologous types as they are visualized in the schema; when, for example, Coccidioides would occupy the position with Endomyces; Saccharomyces would occupy the position with other true yeasts; Gilchrist's blastomycete (under existing knowledge best regarded as an Oidium) would occupy the position with Monilia, and Torula would occupy the position with the homologous nonpathogenic Torula.

This approach of the subject does not offer a perfect solution but it serves a useful purpose, although admittedly temporizing until more certain knowledge is at hand concerning the life cycles of the organisms. It demonstrates a mutual relationship between the organisms and the diseases which they produce, and substitutes an understandable explanation where confusion had existed.

COMMON FEATURES OF BLASTOMYCES AND COCCIDIOIDES; THEIR ULTIMATE CLASSIFICATION

Most of the difficulties have arisen over attempts to classify Blastomyces and Coccidioides. Coccodioides reproducing in the tissues by endogenous sporulation and not by budding is apparently distinctly different from Blastomyces wherein reproduction is assumed to occur only by budding. Some of the attempts which have been made to reconcile the systematic position of these two organisms to existing classifications, or which demonstrate their perfect classification will be cited.

Castellani has taken the position that the so-called spores of Coccidioides are in reality not spores, but represent exaggerations of the granules and spherules common to both Blastomyces and Coccidioides. The sporulation stage of both, according to this opinion, is still to be found. On this assumption he reclassified the organisms of both diseases in a common genus Blastomycoides (of the Fungi imperfecti, Oosporaceae). So far there appears no confirmation of this opinion.

If the organisms of both diseases were of the genus Oidium, as Ophüls and Ricketts held, the problem of classification would be reduced. This, however, so far as Coccidioides is concerned, seems untenable for reasons already given.

Assuming that, as is quite likely true, Coccidioides reproduces in the tissues by endospores, and may be classified as an Ascomycete of the family Endomycetaceae, the perfect classification for that organism has been attained. For Blastomyces the perfect classification is as yet problematic, but certain characteristics which have been reported from time to time tend toward ultimate classification of this fungus in a similar if not identical systematic position. The only distinctive classifiable feature which Coccidioides holds over Blastomyces is that in the former an endosporulating stage has been found. When a similar stage has been discovered for Blastomyces, this essential difference will be removed. Then possibly the two organisms will be found in the same or related genera.

A few observations have been made which tend toward solution of the problem in this way. Ricketts was the first to suggest ascospore production in the organisms of blastomycosis. He observed endocellular bodies in the protoplasm of budding cells in six cases of cutaneous blastomycosis (Cases 3, 6, 7, 8, 9, 10 of his monograph). He proposed that these inclusions might prove to be spores but at the same time commented that final evidence of this was wanting. LeCount's and Myers' case was more convincing, in which the same dual processes of reproduction, as Ricketts had described, were seen. Their descriptions also included the finding of empty spore cases, apparently left behind by rupture and discharge of the spores. Stober made similar observations in one case and also found bodies in one of his cultures resembling ascospores. Montgomery and Ormsby noted bodies which strongly suggested endospores in some organisms, but they were unable to observe their further development. Commenting on this, they stated: "It may be that further study will remove the one fundamental difference between them (blastomycosis and coccidioidal granuloma), that is, the behavior of the organisms in tissue, and prove the conditions to be but varieties of the same process." Wade described a case which exhibited innumerable organisms of small size in the tissues of a case of blastomycosis, but he interpreted their formation to rapid budding of immaturely developed forms. He did not regard the presence of empty "spore sacs" as significant.

Mellon recorded the most direct evidence of sporulation by Blastomyces. He observed the formation of four-celled asci in cultures of the parasites of blastomycosis. On the basis of his observations he suggested that these organisms should be allocated among the Ascomycetes (Endomyces) rather than with the Oidia of Ricketts or the Cryptococci of Vuillemin. Confirmation of this finding is all that is needed for its general acceptance. Thus, the taxonomic position of these organisms would be established.

Vuillemin observed similar reproductive bodies in an organism isolated from a case of thrush, which led him to classify it in the class Ascomycetes as Endomyces albicans. This has been suggested as the perfect stage of Monilia albicans. Castellani also described Endomyces as the etiologic factor in thrush, but believed such a finding is illustrative of plurality of etiologic factors rather than an indication of relationship between them.

With such data forming the basis of opinion, it may be anticipated that eventually the organism of thrush (Monilia), the organism of blastomycosis (Oidium), and the organism of coccidioidal granuloma (Coccidioides) will be classified side by side as Endomycetaceae, closely related to the true yeasts, which are in the neighboring family Saccharomycetaceae. The Torula, by more convincing evidence of their suspected close relationship to the true yeasts, would also be included with the Ascomycetes. Thus their complete life cycle would be demonstrated and the proposal for a simplified classification, of apparently related forms, would reach fulfillment in a perfect classification of each form.

In the case reported, the finding of apparent sporulating bodies and budding forms in the tissues supports the view that Blastomyces and Coccidioides are closely related forms.

REPORT OF CASE

A man, aged forty-one years, was admitted to The Mayo Clinic March 14, 1931. He was born in Germany and immigrated to America at the age of twenty-four. He settled on an Iowa farm and ten years later moved to southern Minnesota, where he also engaged in farming. He had not travelled elsewhere in the United States. About January 1, 1931, he slipped and fell backward, striking his head on a brace. Following this, he appeared to have lost strength and occasionally experienced periods of mild vertigo. February 15, he again struck his head while straightening up from a stooping position. This was followed by headache of frontal and occipital type, which persisted constantly, with periods of great severity. The headache was increased by standing and considerably eased when lying down. About March 1, he began to experience intermittent attacks of vomiting, and noticed a swelling on the inner side of his left leg. Otherwise his past history was essentially negative, except for occasional colds of the thorax with nonproductive cough.

On admission, the patient was somewhat lethargic. Bodily movements were accompanied by vertigo, with some swaying from side to side. Horizontal and vertical nystagmus, and slight lack of coordination of movements were observed. His neck was slightly rigid. Ocular examination revealed the left pupil to be slightly larger than the right; there was beginning edema of the disk, which was more in the left eye than in the right. A subcutaneous abscess, 2.5 cm. in diameter, was present on the mesial aspect of the upper third of the left leg. Spinal fluid, withdrawn under slightly increased pressure (240 mm. of water), was normally clear; the Wassermann reaction of the spinal fluid was negative; a globulin test was positive; total protein was 70 mg. in each 100 c.c.; lymphocytes numbered 29 and polymorphonuclear leucocytes 3 in each cubic millimeter. The gold sol curve was of the zone 1 type. Roentgenograms of the head, thorax and left tibia gave negative results. The Wassermann reaction of the blood was negative. The blood and urine were normal. The blood pressure in millimeters of mercury was 126 systolic and 78 diastolic; the pulse rate was 48 beats each minute and the temperature was 98° F.

During the patient's stay in hospital his condition was about the same as on admission. The outstanding symptoms were rigidity of the neck, nystagmus and vomiting. March 17, 1931, the subcutaneous abscess of the left leg was aspirated and 10 c.c. of pus was obtained. Bacteriologic examination of the pus was negative for Mycobacterium tuberculosis by smears and inoculation of guinea pigs. In brain broth and blood-agar plates there was no growth of microorganisms. The temperature chart revealed an irregular curve, mostly within normal or subnormal range. On three occasions it reached 99° F. and twice it was 99.5° F. The pulse rate ranged between 60 and 70; the respiratory rate was normal. March 27, the abscess of the leg was practically healed and since there was no change in the general progress of the disease which, in spite of repeated tests, would render the diagnosis positive, the patient returned to the care of his home physician. The diagnosis was held to be indeterminate, but there was clinical evidence of an infratentorial intracranial lesion.

At home, the patient's condition remained substantially unchanged. A slight cough developed, which became paroxysmal, dry, and never productive. The abscess of the leg returned, and drained spontaneously through multiple small sinus tracts. The skin over the abscess, except for the minute sinuses, remained intact. A second lesion, similar to the abscess of the leg, appeared over the sacrum, and likewise drained spontaneously. The temperature, pulse rate and respirations, and general mental and physical state remained about as they had been during the patient's stay at the clinic. He died unexpectedly May 14, 1931. Necropsy was performed eighteen hours after death. Vascular embalming had been done. The essential observations were as follows:

Fig. 2.—Multilocular, caseous mass in the lower lobe of the right lung.

Macroscopic Examination.—On the mesial aspect of the head of the left tibia there was a subcutaneous area of suppuration measuring 3 cm. in diameter. It was only slightly elevated, and in its central part was punctured by multiple small sinuses from which seropurulent exudate could be expressed. The suppuration extended to the periosteum but did not involve the bone. A similar lesion was found over the sacrum.

In the upper lobe of the right lung, along the lateral margin, there was an abscess 1.5 cm. in diameter, containing thick purulent material with but slight reaction surrounding it. There were other, firm hemorrhagic nodules measuring 1.5 cm. in diameter, throughout the upper and lower lobes. In the right lower lobe there was a circumscribed, ovoid portion of consolidation; the pleura over the area was hyalinized and thickened. On section this mass measured 6 cm. in diameter and presented the appearance of a multilocular zone of necrosis; the cavities contained caseous material. The margins of this zone were not heavily encapsulated, although condensation and consolidation of the parenchyma of the lung was evident at the periphery, which was intensely hemorrhagic in its outer aspect and tended to be grayish as the zone of necrosis was approached (Fig. 2). Separate

from the large mass, but nearby, there were a few firm grayish-white nodules, 0.5 cm. in diameter, but without central softening. The lower lobe of the left lung exhibited indistinct zones of increased consistence, presenting a slightly nodular appearance, as of bronchopneumonia. Peribronchial lymph nodes were enlarged and on section revealed areas of apparent fibrosis and caseation. Deposits of calcium could be detected in some areas.

The ventral surface of the right lobe of the cerebellum was of firm consistence, with obliteration of the cerebellar convolutions. On section the area had the appearance of a multilocular abscess, containing zones of caseation and other areas of suppuration. There was no evidence of meningitis except locally over the lesion just described. The remainder of the brain appeared normal.

From the macroscopic appearance of the lesions, it was apparent that we were dealing with granulomatous disease. Microscopic examination of pus and caseous material from the lungs mounted in 10 per cent potassium hydroxide revealed numerous doubly contoured refractive bodies.

Histologic Examination.—The microscopic study was made from tissues fixed in Orth's fluid. Because of previous embalming the Zenker fixation for the Mallory stains was substituted by using the Weigert mordant as described by Kernohan. Staining methods included hematoxylin and eosin, scharlach r, Brown-Gram, carbolfuchsin-methylene blue for Mycobacterium tuberculosis, and Mallory's aniline blue as modified by Wade.

Abscess left leg: The stratum corneum revealed an abnormal hyperconification with overlying crusts consisting of cellular débris, leucocytes and fibrin. In places, apparently corresponding to the small sinus tracts, the dermis and epidermis were separated by ulceration and sloughing. Adjacent to these tracts there was some extension of the ulceration, and granulation tissue arising from the dermis formed the base of the ulcer. The epithelium at the borders of the ulcer was hyperplastic. The hyperplastic reaction also involved the epithelium of some of the hair follicles. In the deeper layers of the dermis there were clusters of epithelial cells exhibiting a pseudopearl formation or presenting necrotic or abscess centers. Corresponding to the epidermal hyperplasia, the dermal papillae were of irregular morphology, revealing edema, congestion and leucocytic infiltration. The cellular collections predominated with polymorphonuclear leucocytes, among which were many basophils, but regularly present were lymphocytes, large monocytes and plasma cells. Giant cells were also conspicuous. Small abscesses or diffuse cellular reactions were the rule rather than tubercles. Lying free in the tissue spaces, or contained within giant cells, large numbers of ovoid or spherical cells were found. These presented a double refractile outer zone and a clear central protoplasm or one in which from five to six acidophilic or basophilic granules appeared. The granules were central or peripheral. Some forms contained refractive unstained bodies in their cytoplasm, usually from four to six being found. Budding could not be ascertained with certainty, although a few forms presented knob-like swellings on one side, and others appeared as pairs of cells.

Lung: What appeared grossly as a multilocular granuloma, was confirmed. Preparations from the large multilocular area revealed the cavities to be filled with necrotic detritus, having a hyaline appearance and staining diffusely with eosin in the hematoxylin and eosin preparations. In the hyaline material, well formed cells

were rarely seen, only an occasional lymphocyte or refractive yeast-like form being observed. At the periphery of the multilocular portions basic staining cellular débris blended gradually into the living reaction zone. In the zone of tissue reaction there was granulation tissue composed of innumerable vascular channels, supported in a tissue framework of fibroblasts and endothelioid cells. Lymphocytes, monocytes, and occasional plasma cells and polymorphonuclear leucocytes were in abundance. Giant cells were also fairly numerous. Farther away from the large necrotic zone, daughter lesions were present, apparently as outgrowths from the older lesions. They were in the form of tubercles, with endothelioid and giant cells prominently displayed. Within the giant cells and lying in the interstices of the tissue there were numerous yeast-like forms.

Peribronchial lymph nodes: The lesions in the peribronchial lymph nodes were similar to those in the lung. Formation of tubercles with central necrosis and peripheral granulomatous reactions predominated the picture (Fig. 3). The

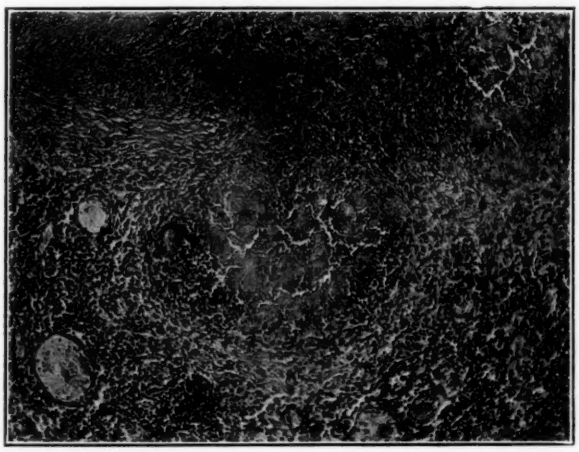


Fig. 3.—Tubercle formation with central necrosis in a peribronchial lymph node (hematoxylin and eosin).

necrosis was perhaps more extensive than elsewhere, as also were the daughter tubercles. The great resemblance of the tubercles to those of tuberculosis was also a noteworthy feature. Yeast-like cells appeared free in the necrotic centers, lying in the peripheral exudate and granulation tissue and within the giant cells.

Cerebellum: In the cerebellum the lesions presented central necrosis in which cellular detritus was visible here and there. At the periphery of the necrotic lesions collections of polymorphonuclear leucocytes were encountered which were, in some areas, aggregated as miliary abscesses. The encapsulations of both the larger necrotic areas and the miliary abscesses were of granulomatous tissue, consisting of young connective tissue cells, endothelioid cells and small blood vessels, and in which lymphocytes and other leucocytes were collected. Giant cells were also prominent (Fig. 4). In the necrotic areas few refractive yeast-like cells were observed, but in the miliary abscesses and granulomatous tissue these fungi were very numerous, either lying free in the exudate or in the granulomatous areas of reaction, or were contained within the cytoplasm of the giant cells (Fig. 5). The cerebellar tissue which surrounded the zone of inflammation was not immediately

normal but revealed necrosis and compression grading gradually to the normal, evidencing the progressive character of the lesion as it extended peripherally into the previously uninvolved tissues.

The organisms: The organisms in the cerebellar lesion were sufficiently distinctive to warrant a more detailed description. They usually appeared as spherical cells; a few, however, were oval, and with hematoxylin and eosin staining they exhibited the customary double contoured highly refractive capsule. The endo-

Fig. 4.—Confluent tubercles in the cerebellum showing giant cells and the surrounding cellular collections and vascularity (hematoxylin and eosin).

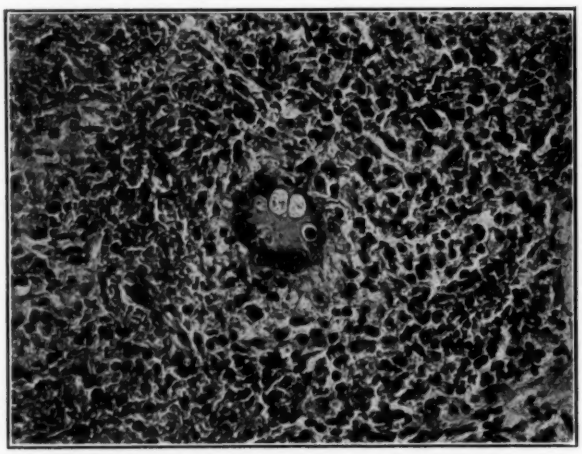


Fig. 5.—Organisms enclosed within a giant cell from the cerebellum (hematoxylin and eosin).

plasmic portion was revealed as either unstained, faintly basic stained and homogeneous, or granular, sometimes containing ill-defined vacuolated bodies. The size of the cells was quite variable, the predominating measurement being 10 microns with limits from 3 to 25 microns. By use of the aniline-blue staining technic, the capsule and protoplasmic portions of the cells were revealed in much greater detail. The capsule appeared as a homogeneous, basic staining zone with slightly more deeply staining outer and inner membranes. It varied in thickness proportionately with the size and age of the cells. Sometimes there appeared to be an adventitious

capsule. In most instances a clear, unstained refractive space was present immediately within the capsule, and in others this space was occupied by a condensation of finely divided basic staining cytoplasm which sometimes was dotted by acidophilic or deeply basophilic granules. In other cells the cytoplasm was similar, but was distributed uniformly and left neither outer clear zone nor central vacuolization. The cytoplasmic granules were often small and basic staining or larger and more

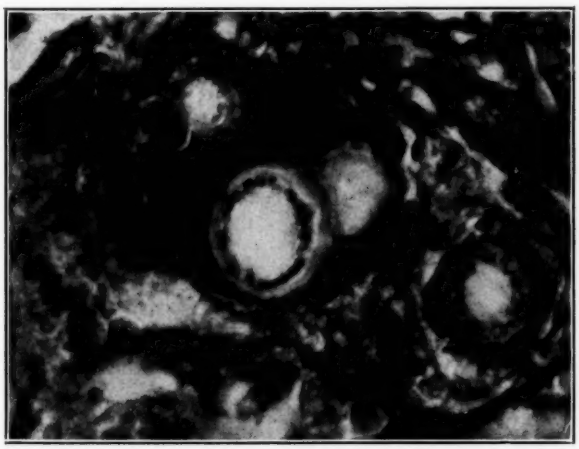


Fig. 6.—Organism with thick capsule and three large refractile bodies in a nearly homogeneous cytoplasm (Mallory's aniline blue).

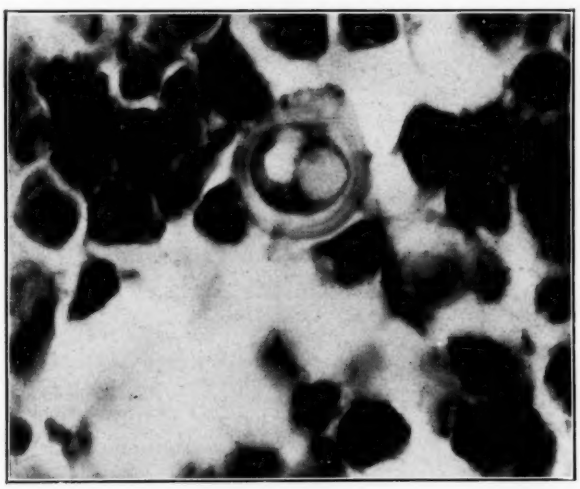


Fig. 7.—Two organisms with a single large central vacuole surrounded by a zone of granular spore-like bodies (Mallory's aniline blue).

clearly defined deeply basophilic or acidophilic bodies. The granules were larger and more numerous in the larger cells. The number of granules varied from one or two up to ten or more (Fig. 6). In the largest cells, the center of the granule was sometimes vacuolated and refractive. In addition to the granules, refractive spherules were frequently seen in the cytoplasm; large ones as already described for the central areas and also multiple smaller ones (Fig. 7). The number present was inconstant, there being frequently two, four or more, to innumerable ones. It

was impossible to stain these refractive spherules by either acid-base dyes or scharlach r, but in some there was a more deeply staining cytoplasm immediately surrounding the sphere. In some cells the granules were regularly arranged in rosette forms. To add further proof that these were endospores and not pseudospores it would be necessary to describe their further evolution. A few empty capsules could be found, which appeared as if the cell had discharged its contents by rupture.

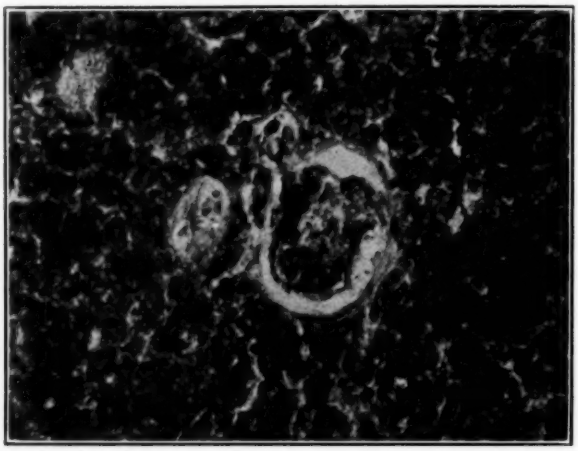


Fig. 8.—A large organism filled with heavy granules, with rupture of capsule and apparent evacuation of spore-like bodies into surrounding tissue (Mallory's aniline blue).

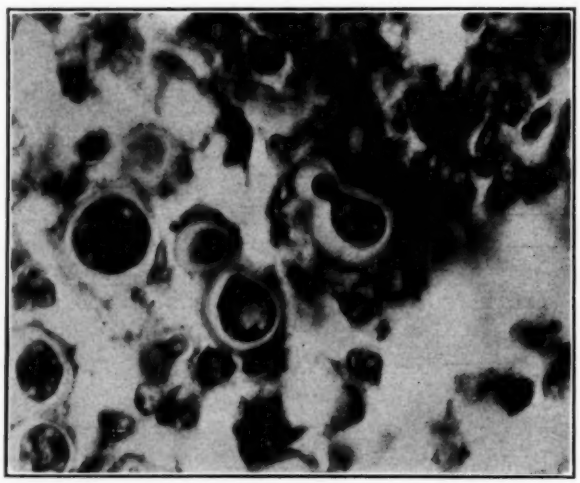


Fig. 9.—Well developed budding form (Mallory's aniline blue).

The actual liberation of spores from the ruptured cell was not observed with certainty, though in a few instances spore-like granules could be seen having the appearance of being extruded from the ruptured cell (Fig. 8), and clusters of very small yeast-like cells were occasionally found.

The preparation stained with aniline-blue, besides revealing the spore-like forms, presented, as in the other lesions of the case, a few forms apparently in the various stages of budding. As revealed in the various microscopic fields, the earliest evidence of budding consisted of condensation of the inner cytoplasm into a

small knob-like excrescence, occupying a position in the peripheral refractive clear space. Earlier there was no change in the heavy outer capsule. In the enlarged buds, however, the capsule was compromised, apparently to make way for this growth. Daughter cells were thus formed, appearing exactly as the parent, in miniature (Fig. 9). Finally they were completely separated and surrounded by an independent capsule. The same appearance of protoplasmic granules, spore-like spherules and vacuoles could be seen in the apparent budding forms as in the nonbudding forms (Fig. 10). It could be possible that these forms, rather than representing stages of budding, were preparing for rupture of the spore with liberation of the endospores; this, however, seems improbable in view of the successive stages of budding which were encountered.

Stains for Mycobacterium tuberculosis were negative in all of the lesions.

Comment.—The classification of the parasite in this case was rendered more difficult because opportunity to study the cultural characteristics of the organism was lost by embalming previous to necropsy. It was necessary, therefore, for purposes of classification, to rely entirely on the details of the lesions and the morphologic features of the organism.

Fig. 10.—Organisms with vacuoles and spore-like bodies in cytoplasm. Two show early budding (Mallory's aniline blue).

The diagnosis of torulosis was first and most readily excluded. In torulosis the organisms occur in zoogleal masses due to a gelatinous matrix; there was no suggestion of this in our case. That the disease was not saccharomycosis of the Busse-Buschke or Curtis type could not be dismissed so easily. The cells were generally larger than have been described in the true yeast infections. The extreme rarity of the true yeast infection was also opposed to its classification as such.

The classification then rested between blastomycosis or coccidioidal granuloma. From the clinical features of the disease and from the gross and microscopic pathologic characteristics, the diagnosis of coccidioidal granuloma is strongly suggested. The short duration of symptoms, the marked resemblance of the lesions to tuberculosis, and the extensive involvement of the peribronchial lymph nodes are more nearly like coccidioidal granuloma than blastomycosis. The diagnosis of

coccidioidal granuloma receives added support from the morphology of the parasite in the tissues, especially from the occurrence of the spore-like bodies. Budding, however, is considered to be a condition which negates such a diagnosis. Nevertheless, since the greatest tendency of both the organism and the disease in our case was toward coccidioidal granuloma, we have elected that as a presumptive diagnosis.

In consideration of the occurrence of apparent endogenous sporulation and budding by the same organism it has been necessary, as indicated by our term "presumptive diagnosis" to refer to this as a case of coccidioidal granuloma with some reservation, but since we have elected the position that all organisms of this group are probably mutations of a more highly developed form such as Endomyces, various morphologic combinations could occur, thus rendering an exact systematic classification impossible when they do not conform with so-called standard concepts. These organisms, so far as our knowledge of them and homologous nonpathogens is developed, may assume various modes of growth and biologic reactions, depending on environmental influences, as previously noted.

The diagnosis of coccidioidal granuloma has hitherto, to our knowledge, never been made in residents of Minnesota. More than 80 per cent of the known cases have originated in California. This adds considerable interest to this report, indicating that an organism capable of evoking a disease similar to that ordinarily endemic in California may also be found in Minnesota. That its occurrence in Minnesota and elsewhere outside of California should be anticipated is indicated from isolated reports from other localities where occasional cases of coccidioidal disease have developed in patients who had never been in California, and from knowledge of the biology of the group as previously considered.

Nothing could be determined regarding the source of the infection in our case. No other cases were known in the locality from which the patient came. The primary lesion was possibly pulmonary, with lymphatic dissemination to the peribronchial lymph nodes, and hematogenous distributions to the cerebellum and cutaneous tissue. The lesion on the left leg, the other possible primary, appeared after the cerebellar disturbance became manifest. It also resembled closely an embolic lesion, in that it originated as a subcutaneous abscess without exhibiting extensive ulcerative or verrucous characteristics.

SUMMARY AND CONCLUSIONS

An unusual case of systemic infection due to yeast-like parasites has been presented. The case is of particular interest because of its organic localizations, which were cerebellar, pulmonic and subcutaneous (two small abscesses). Its clinical, etiologic, and pathologic resemblance to coccidioidal granuloma in a resident of Minnesota, who had not recently travelled elsewhere, is also noteworthy. In this case, the organisms revealed dual processes of reproduction in the tissues, namely, by budding and sporulation, such as has been demonstrated by Ricketts, Stober, Montgomery, LeCount and Myers, and others. The literature on the subject of infections due to yeasts and yeast-like organisms has been partly reviewed. The review demonstrates that a close relationship exists between the various yeasts and yeast-like pathogens and the diseases which they cause, and that attempts to classify without keeping this relationship in mind tends to confuse rather than clarify the subject.

REFERENCES

1. Buschke, A.: Die Sprosspilze. In: Kolle, W., and von Wassermann, A.: Handbuch der pathogenen Mikroörganismen, Jena, Gustav Fischer, **5**: 155-210, 1913.
2. Busse, Otto: Ueber parasitäre Zelleinschlüsse und ihre Züchtung, Centralbl. f. Bakteriol. **16**: 175-180, 1894.
3. Busse, Otto: Ueber Saccharomycosis Hominis, Virchow's Arch. f. path. Anat. u. Physiol. **140**: 23-46, 1895.
4. Castellani, Aldo: Fungi and Funguous Diseases, Chicago, American Medical Association, p. 203, 1928.
5. Castellani, Aldo: Blastomycosis and Some Other Conditions Due to Yeast-Like Fungi (Budding Fungi), Am. J. Trop. Med. **8**: 379-422, 1928.
6. Curtis, F.: Sur un parasite végétal de l'èspece des levures, Presse méd. **3**: 370-371, 1895.
7. Curtis, F.: Contribution à l'étude de la saccharomycose humaine, Ann. de l'Inst. Pasteur **10**: 449-468, 1896.
8. Greenfield, J. G.: Blastomycotic Infection of the Nervous System, Med. Sc. Abstr. & Rev. **10**: 267-273, 1924.
9. Greenough, R. B.: Cell Inclusions in Cancer and in Non-Cancerous Tissue, J. Med. Res. **7**: 360-382, 1902.
10. Gilchrist, T. C.: A Case of Blastomycetic Dermatitis in Man, Johns Hopkins Hosp. Rep. **1**: 269-283, 1896.
11. Gilchrist, T. C., and Stokes, W. R.: The Presence of an Oidium in the Tissues of a Case of Pseudo-Lupus Vulgaris (Preliminary Report), Bull. Johns Hopkins Hosp. **7**: 129-133, 1896.
12. Gilchrist, T. C., and Stokes, W. R.: A Case of Pseudo-Lupus Vulgaris Caused by a Blastomyces, J. Exper. Med. **3**: 53-78, 1898.
13. Guilliermond, Alexandre, and Tanner, F. W.: The Yeasts, New York, John Wiley and Sons, 1920, pp. 424.
14. Henrici, A. T.: Molds, Yeasts and Actinomycetes, New York, John Wiley and Sons, 1930, pp. 296.
15. Kernohan, J. W.: A New Modification of Mallory-Heidenhain's Differential Staining Method and Adaptation of Formalin-Fixed Material for Mallory's Stains, Am. J. Clin. Path. **1**: 399-403, 1931.
16. LeCount, E. R., and Myers, J.: Systemic Blastomycosis, J. Infect. Dis. **4**: 187-200, 1907.
17. MacNeal, W. J., and Taylor, R. M.: Coccidioides Immitis and Coccidioidal Granuloma, J. Med. Res. **30**: 261-274, 1914.
18. McGehee, J. L., and Michelson, I. D.: Torula Infection in Man: Report of a Case, Surg. Gynec. Obst. **42**: 803-808, 1926.
19. Mellon, R. R.: Studies in Microbic Heredity. VI. The Infective and Taxonomic Significance of a Newly Described Ascospore Stage for the Fungi of Blastomycosis, J. Bacteriol. **11**: 229-252, 1926.
20. Montgomery, F. H., and Ormsby, O. S.: Systemic Blastomycosis, Arch. Int. Med. **2**: 1-41, 1908.
21. Montgomery, Hamilton: Systemic Blastomycosis, Med. Clin. N. Amer. **14**: 651-662, 1930.
22. Nichols, E. H.: The Relation of Blastomycetes to Cancer, J. Med. Res. **7**: 312-359, 1902.
23. Ophüls, W.: Further Observations on a Pathogenic Mould Formerly Described as a Protozoon (Coccidioides Immitis, Coccidioides Pyogenes), J. Exper. Med **6**: 443-485, 1901-1905.
24. Ophüls, W., and Moffitt, H. C.: A New Pathogenic Mould (Formerly Described as a Protozoon: Coccidioides Immitis Pyogenes), Philadelphia Med. J. **5**: 1471-1472, 1900.
25. Popoff, Leo: I. Untersuchungen über die Wirkung der Bierhefe und der in der Pasteur'schen Flüssigkeit enthaltenen Organismer auf der thierischer Körper, Berl. klin. Wchnschr. **9**: 513-516, 1872.
26. Rabinowitsch, Lydia: Untersuchungen über pathogene Hefearten, Ztschr. f. Hyg. u. Infectionskrankh. **21**: 11-24, 1896.
27. Ricketts, H. T.: Oidiomycosis (Blastomycosis) of the Skin and Its Fungi, J. Med. Res. **6**: 373-546, 1901.
28. Rixford, Emmet, and Gilchrist, T. C.: Two Cases of Protozoon (Coccidioidal) Infection of the Skin and Other Organs, Johns Hopkins Hosp. Rep. **1**: 209-265, 1896.
29. Roncali, D. B.: Die blastomyceten in den sarkomen, Centralbl. f. Bakteriol. **18**: 432-434, 1895.
30. Sanfelice, Francesco: Ueber einen neuen pathogenen Blastomyceten welcher innerhalb der Gewebe unter Bildung kakartig ausehender Massen degeneriert, Centralbl. f. Bakteriol. **18**: 521-526, 1895.
31. Sanfelice, Francesco: Ueber eine für Tiere pathogene Sprosspilzart und über die morphologische Uebereinstimmung, welche sie bei ihrem Vorkommen in der Geweben mit den vermeintlichen Krebscoccidienzeigt, Centralbl. f. Bakteriol. **17**: 113-118, 1895.
32. Sanfelice, Francesco: Ueber die pathogene Wirkung der Sprosspilze zugleich ein Beitrag zur Aetiologie der bösartigen Geschwülste, Centralbl. f. Bakteriol. **17**: 625-634, 1895.

33. Sanfelice, Francesco: Sull' azione patogena dei blastomiceti, Policlinico (sez. chir. **2**: 204-211, 1895.
34. Sanfelice, Francesco: Ueber Immunität gegen Blastomyceten, Centralbl. f. Bakteriol. **20**: 219-222, 1896.
35. Stober, A. M.: Systemic Blastomycosis. A Report of Its Pathological, Bacteriological and Clinical Features, Arch. Int. Med. **13**: 509-556, 1914.
36. Stoddard, J. L., and Cuttler, E. C.: Torula Infection in Man. Monograph of the Rockefeller Institute for Medical Research, pp. 1-98, Jan. 31, 1916.
37. Vuillemin, P.: Quoted by Guilliermond, Tanner, Castellani, Mellon and Henrici.
38. Wade, H. W.: A Variation of Germation of Blastomyces Dermatitis in the Tissue Lesion, J. Infect. Dis. **18**: 618-629, 1916.
39. Walker, J. W., and Montgomery, F. H.: Further Report of a Previously Recorded Case of Blastomycosis of the Skin; Systemic Infection With Blastomyces; Death; Autopsy, J. A. M. A. **38**: 867-871, 1902.
40. Weis, J. D.: Four Pathogenic Torulae (Blastomycetes), J. Med. Res. **7**: 280-311, 1902.
41. Wernicke, Robert: Ueber einen Protozoenbefund bei Mycosis Fungoides (?), Centralbl. f. Bakteriol. **12**: 859-861, 1892.
42. Wolbach, S. B.: The Life Cycle of the Organism of "Dermatitis Coccidioides," J. Med. Res. **13**: 53-60, 1904.

SULPHEMOGLOBINEMIA*

JAMES C. HEALY, M.D., BOSTON, MASS.

SULPHEMOGLOBINEMIA may be definied as a condition in which the normal blood pigment hemoglobin is replaced in part by sulphemoglobin. It is considered to be a rare condition, only 38 cases having been reported in the literature, 15 from the United States, the remainder from abroad. The authors feel however that it is much more common and that the diagnosis is frequently overlooked especially in those cases where the symptoms are mild. The two cases in this review were discovered accidentally while in consultation for a totally unrelated symptom.

The literature relating to sulphemoglobinemia is meager. In 1902 Stokvis[1] reported a case of cyanosis associated with a chronic dysentery and without cardiac or pulmonary complications. On examination of the blood he found an absorption band in the red portion of the spectrum in addition to the normal bands of oxyhemoglobin. This he called methemoglobin but for reasons described later in this paper we believe that it was sulphemoglobin. He dignified the condition with the title "Enterogenous Cyanosis" and assumed that absorption of toxins in this case of chronic dysentery was sufficient to produce the abnormal pigment. In the same year Talma[2] described two similar cases of cyanosis, one of which was accompanied by a diarrhea. In his report he described the same absorption band in the red part of the spectrum. Further he ascertained that the abnormal pigment resided in the red corpuscles and not the plasma.

Three years later van den Bergh[3] while studying 2 cases of enterogenous cyanosis found that one was caused by methemoglobin the other by sulphemoglobin. This was the first recognition of the clinical entity, sulphemoglobinemia. The distinction was made by spectroscopic examination of the blood and the theory of aniline derivative ingestion as a cause of methemoglobinemia was established by him.

---

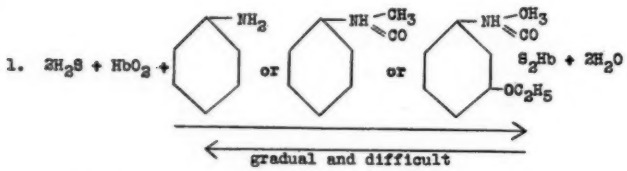
*From the Department of Pharmacology, Tufts College Medical School.
Received for publication, December 24, 1931.

The pathogenesis of sulphemoglobinemia has been studied intensively for the past twenty years and several probable theories have been evolved. Lewin[4] before the recognition of such a condition described the absorption of H_2S after its oral, subcutaneous or intravenous administration and proved its presence in the blood and other tissues. Meyer[5] in a similar manner detected sulphides in the blood after rectal introduction of dihydrogen sulphide. Clarke and Hurtley[6] in 1907 showed that the presence of powerful reducing agents such as the nitrites together with dihydrogen sulphide in the blood caused sulphemoglobinemia. Later Wallis[7] studying this condition found a nitrite producing bacillus in the mouths of affected persons, the absorption of nitrites formed in this way being sufficient to cause reduction. Similar organisms were obtained from cultures of the stools. Several other workers following the same technic have been unable to substantiate his claims. In 1925, Snapper[8] showed by clinical and experimental means that one of the aniline derivatives, acetphenetedin, in large doses would produce sulphemoglobinemia. Furthermore he found that methemoglobin was formed with great difficulty in those patients and animals taking aniline derivatives. The feeding of sulphur by mouth hastened the formation of sulphemoglobin. He elaborated a theory that hemoglobin is sensitized by the aniline and can unite with sulphides absorbed from the intestine to form the new pigment.

This appears to be a fair conclusion for unless the hemoglobin were changed in some way by an exogenous agent the condition would be protean. Ten per cent of ingested sulphur is absorbed and, taking into consideration a limited intake and measured output, the difference in urinary sulphur is due to the oxidation products of dihydrogen sulphide formed in the intestine by bacterial action.[9] Denis and Reed[10] have proved further that dihydrogen sulphide introduced into the intestine is readily absorbed and causes a change in the respiratory rate in a few minutes. They detected the gas in the blood and ascribed its fate to oxidation to sulphuric acid and sulphates. Fortunately most of the dihydrogen sulphide formed in the body is located in the colon, the site of bacterial action, for these same authors found the absorption rate much greater in the small intestine.

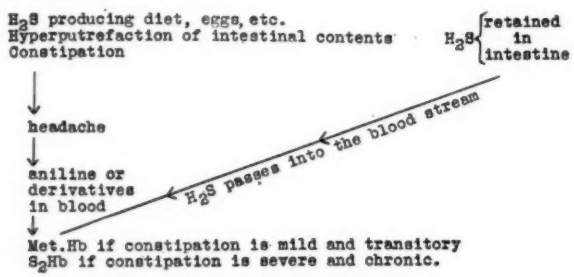
Since the absorption of dihydrogen sulphide from the intestine in a concentration above that which the organism can oxidize and eliminate easily is necessary to cause the condition, one should expect either a large dihydrogen sulphide production or an obstruction to fecal outlet. This latter fact has been proved experimentally and clinically, a majority of the patients having been affected with severe and persistent constipation. In connection with Snapper's theory of hemoglobin sensitization to sulphur by means of aniline it is interesting to note the work of Harrop and Waterfield.[11] They produced sulphemoglobinemia in splenectomized dogs by feeding sulphur without an aniline derivative. This suggests some alteration in the hemoglobin molecule which allows union with sulphur after splenectomy, and suggests a possible splenic dysfunction in those cases of sulphemoglobinemia which have a history of no drug ingestion. Mason and Conroy[12] reviewing thirteen cases failed to find any evidence of coal tar product medication. To acetphenetedin, Harrop and Waterfield[11] add acetanilid and exposure to aniline as etiologic agents. In both of our cases the habitual and excessive use of bromoseltzer was the factor.

Diagrammatically the blood pigment changes are as follows:



2. $2H_2S + HbO_2$ —— aniline alone ——→ (S_2Hb) + $2H_2O$ (MetHb)

3. In absence of H_2S
   a. aniline, small dose, easily ——→ Met Hb
   b. acetanilid, larger dose, readily ——→ Met Hb
   c. acetphenetidin, larger dose, with difficulty ——→ Met Hb
4. In presence of H_2S, all three produce S_2Hb
5. The oxygen carrying power of the blood is not reduced and may be normal or above normal.
6. Both diseases are caused as follows:


H2S producing diet, eggs, etc.
Hyperputrefaction of intestinal contents
Constipation
H2S
retained in intestine
headache
aniline or derivatives in blood
H2S passes into the blood stream
Met.Hb if constipation is mild and transitory
S2Hb if constipation is severe and chronic.

REPORT OF CASES

CASE 1.—A. C., a male accountant, fifty years of age, was seen on October 20, 1931, complaining of pain in the left side of the chest. A diagnosis of pleurodynia was made and treatment gave prompt relief.

A day later a history of addiction to acetanilid was obtained. Six years ago the patient's wife died after a long illness and during that period he was obliged to do considerable of his office work at night. He had frequent frontal headaches after a week, more noticeable at night and relieved by rest of the eyes. Realizing the association of eyestrain to headaches he planned to see an oculist but on account of business pressure postponed the examination and began to take bromoseltzer for relief. At first he averaged two doses or about 5 grains of acetanilid a night and in a few weeks took as much as 10 doses a day. The headaches became generalized and it gradually became necessary to take a full dose of the drug every hour to insure comfort, the patient always carrying a medium sized bottle of bromoseltzer in his pocket for use when unable to go to a soda fountain. Six months later he had his eyes refracted and glasses changed but the headaches persisted. They were never severe enough to keep him awake but on arising he was conscious of the discomfort and would take a generous dose of bromoseltzer before breakfast. Immediately the headache subsided and then recurred in an hour necessitating more of the drug, and so on for the rest of the day.

Two or three times he had made sincere attempts to break the habit but irritability, sleeplessness, inability to concentrate on his work, an intense desire for a salty taste in his mouth and the headache would destroy his resolutions. Peculiarly enough, in the later stages the first three of the above symptoms were the ones which influenced him greatly.

The past history was entirely negative except for severe constipation since childhood, the control of which necessitated a daily enema or purge.

On physical examination the skin was found to be pale and dry, the mucous membranes of mouth cyanosed, as were also the nail beds. Aside from pyorrhea alveolaris and general hyperactive reflexes the examination was negative.

Urine was normal.

Blood examination: Red cell count, 5,200,000, hemoglobin 88 per cent by the Sahli method, white cell count 9400, morphology of corpuscles and differential count were normal. Spectro-

scopic examination of the blood revealed the typical absorption band of sulphemoglobin and the other typical reactions outlined below.

A diagnosis of sulphemoglobinemia was made and a diet outlined. Eggs were restricted and a high fat intake prescribed. Acting on Long's[15] observations, inhalations of oxygen and carbon dioxide were given the first day at six hour intervals, 3 treatments in all, each lasting twelve minutes in which time the respiratory rate rose to 36 per minute. On the second day the same number of inhalations were given but the time interval required to reach a rate of 36 respirations a minute was decreased in the latter part of the day to nine minutes. The cyanosis was scarcely apparent on the third day of treatment but the spectroscopic test for sulphemoglobin was still positive. The duration of oxygen and carbon dioxide inhalation was decreased steadily to two minutes on the fifth day, at which time the cyanosis was absent and the test for sulphemoglobin was negative. During the period of treatment ammonium chloride was administered in 15 grain doses four times a day, and the patient abstained from bromoseltzer. To ensure catharsis a half ounce of magnesium sulphate was given by mouth. Small doses of codeine were sufficient to control the headache.

Two days after the cessation of treatment the headache disappeared and for the past two months he has had no recurrences. Frequent spectroscopic examinations of the blood have failed to show the presence of sulphemoglobin.

CASE 2.—H. W. G., a forty-year-old male confectioner was first seen in November, 1931. At that time he came in complaining of sugar in the urine, the glycosuria having been discovered during a route insurance examination. Previously he had been in excellent health and except for frequent headaches the past history was entirely negative. The patient did not volunteer the information about his headaches and this was obtained only by questioning. For these he had used bromoseltzer and had been accustomed to take from 4 to 6 doses of the drug a day. This had gone on for several years and the patient was unable to say whether his symptoms antedated the habit. He had been chronically constipated "all his life" and took 3 alophen tablets before retiring each day. He admitted unnatural irritability for the past year, some insomnia, a tendency to become upset by trivial affairs, and complained of the changed attitude of his family and acquaintances.

Physical examination revealed a well developed and well nourished man apparently in excellent health. The skin and mucous membranes were thought to be normal in color at the first examination, but later, after the blood tests, a slight mauve-lavendar cyanosis was noticed on the lips. Otherwise there was no evidence of pathology.

The urine contained 0.8 per cent of sugar and a twenty-four hour specimen contained 16 gm. Fasting blood sugar was found to be 140 mg./100 c.c. and a sugar tolerance test after the ingestion of 100 gm. of glucose gave the following results: 131/100 and 186 mg./100 c.c. On account of the history of bromoseltzer addiction, a spectroscopic examination of laked blood was done and it revealed sulphemoglobin.

Treatment as in Case 1 was instituted with practically identical results in the length of time of inhalations and the decreasing tolerance to CO_2. The glycosuria was absent on the second day, and at the end of a week both headache and sulphemoglobinemia were absent. The patient returned to his regular diet but the glycosuria failed to reappear. A fasting blood sugar on the tenth day was found to be 96 mg./100 c.c., and a sugar tolerance test proved to be normal a day later. Daily urinalysis and one blood sugar test have been found to be normal one month since treatment was discontinued.

DIAGNOSIS

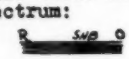
Headache is the most common single symptom as reported in the literature and occurred in both our cases. Our patients described it as a dull oppressive pain, generalized, present at all hours of the day and night, and relieved by bromoseltzer for about an hour.

Constipation in both cases was severe and of long duration. Each patient volunteered the information that a purgative was required every day to insure defecation. In the reported cases this has been a consistent symptom.

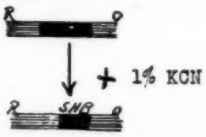
Cyanosis in the absence of other symptoms or signs of heart or lung disease is a characteristic finding in these cases. The cyanosis is mauve-lavendar as described by Harrop and Waterfield[11] and is due to the abnormal pigment in the blood.

Nervous symptoms were marked in both cases. Patient I was definitely of a neurotic type and recognized by his neighbors as "peculiar." For several years he had made himself a burden to relatives and neighbors because of his many manifestations of emotional and nervous instability.

The only single method of making the diagnosis is by spectroscopic examination of the blood, either by means of a hand spectroscope through the lobe of an ear or directly viewing the diluted and laked blood in a tube. In this way all parts of the spectrum except the red and orange are cut out and the band of sulphemoglobin can be seen slightly nearer the orange than the band of methemoglobin. The disappearance of the band after the addition of ammonium sulphide T. S. to the laked blood indicates that the abnormal pigment is methemoglobin. Its persistence confirms the diagnosis of sulphemoglobinemia. The diagnosis of sulphemoglobinemia may be tabulated as follows:

S_2-Hb-aemia	Met-Hb-aemia
a. mauve-lavendar cyanosis, due to mauve-lavendar blood.	a. blue-chocolate-brown cyanosis, due to the same color of the blood.
b. weeks required for cyanosis to fade away gradually upon discontinuance of the drug.	b. forty-eight hours required for the cyanosis to disappear entirely upon discontinuance of the drug.
S_2-Hb-aemia	Met-Hb-aemia
c. spectroscopic tests:	c. spectroscopic tests
1. by pocket spectroscope see light thru ear:	1. same way:
2. draw a little blood from ear and dilute with water in a small tube. Examine the spectrum: 	2. same way:
3. if dilute $(NH_4)_2S$, 1% KCN are added to the above diluted blood:	3. same way:
4. if CO is bubbled thru the original diluted blood:	4. same way:
5. measure the wave-length: 620-607	5. measure the wave-length: 618-630

6. When both S_2-Hb and Met-Hb are present the two absorption bands overlap. Divide the diluted blood into two tubes and add to one of them a drop of 1% KCN. It is the band of Met-Hb that disappears.



Jameson[13] described a spontaneous and rapid cure of the condition, without treatment the case being of unknown etiology. Van den Bergh's case which we may consider to be sulphemoglobinemia was cured by operative treatment of a rectal stricture. Wallis used an autogenous nitroso-bacillus vaccine successfully and Garrod[14] reported satisfactory results in the treatment of a combined methemoglobinemia and sulphemoglobinemia by the same type of vaccine and withdrawal of drugs. In the latter case the cyanosis disappeared after a month of treatment. Long and Spriggs[15] used oxygen inhalations during a fainting attack in one of these cases and noticed the improvement in the cyanosis. All authors advise purgatives, withdrawal of any offending drugs, a high carbohydrate diet, and a low sulphur intake.

To these the author wishes to add the use of oxygen and carbon dioxide inhalations supplemented by a high fat diet and the administration of ammonium chloride by mouth, 15 grains four times a day. The ordinary cylinders used by anesthetists containing 5 per cent carbon dioxide in pure oxygen and a mask of the type used in nitrous oxide work is the only apparatus required. The inhalations were given two or more times a day and continued until the respiratory rate reached thirty-six and the respirations were definitely hyperpneic, or until signs of CO_2 poisoning became apparent. As the cyanosis diminished, the tolerance to carbon dioxide decreased and on the last day of treatment the inhalations could be given for only two minutes.

The prophylactic treatment is important and embraces a policy of educating the public in the dangers of these drugs and a policy of conservatism in prescribing acetphenetedin and acetanilid.

CONCLUSIONS

1. A brief review of the literature and the theories of the pathogenesis of sulphemoglobinemia is presented.
2. Two cases with typical symptoms and signs of the condition are described.
3. A new method of treatment, inexpensive, and simple of application, which shortens the curative period from weeks to days is described.

The author wishes to express his appreciation to Mr. John D. A. Gatsos, A.B., for his assistance in the preparation of this article.

REFERENCES

1. Stokvis, B. J.: Zur Casuistik der autotoxischere enterogenen Cyanosen von Leyden's Festschrift, Berlin **1**: 597, 1902.
2. Talma, S.: Intraglobulare Methamoglobinamie beim Menschen, Berl. klin. Wchnschr. **39**: 865, 1902.
3. Van den Bergh, A. A. H.: Enterogene Cyanose, Deutsches Arch. f. klin. Med. Leipz. **83**: 86, 1905.
4. Lewin, L.: Ueber die Veranderungen des Natrium sulphontimoniats im thierschen Organismes und die Einwiskung des Schwefelwasserstoffes auf das libende Blut, Virchows Arch. f. path. Anat. **74**: 220, 1878.
5. Meyer: Ueber das vechalten und der Nachivers des Schevefelwasserstoffes im Blut., Arch. f. exper. Path. u. Pharmakol. **61**: 325, 1898.
6. Clarke, T. W., and Hurtley, W. H.: On Sulphemoglobinemia, J. Physiol. **36**: 62, 1907.
7. Wallis, R. L. M.: On Sulphemoglobinemia, J. Physiol. **36**: 62, 1907.
8. Snapper, I.: Phenacetin als Ursache fur Sulphaemoglobinamie, Deutsche med. Wchnschr. **51**: 648-650, 1925.
9. Denis, W., and Reed, L.: Concerning the Effect Produced by the Administration of Sulphur on Concentration of Certain Sulphur Compounds in Blood and Urine, J. Biol. Chem. **73**: 51, 1927.

10. Denis, W., and Reed, L.: Action of Blood on Sulfides, J. Biol. Chem. **72**: 385, 1927.
11. Harrop, G. A., and Waterfield, R. L.: Sulphemoglobinemia, J. A. M. A. **95**: 647-650, 1930.
12. Mason, V. R., and Conroy, F. D.: Sulphemoglobinemia, Bull. Johns Hopkins Hosp. **32**: 391-394, 1921.
13. Jameson, R. A.: An Observation on Methemoglobinemia and Sulphemoglobinemia, Quart. J. Med. Oxford **12**: 86, 1918.
14. Garrod, L. A.: On Sulphemoglobinemia, Quart. J. Med. Oxford **19**: 86-94, 1925-26.
15. Long, W. C., and Spriggs, E. I.: On Sulphemoglobinemia, Quart. J. Med. **11**: 102, 1918.

TARTRATE METABOLISM*

I. Grape Tartrates and the Acid-Base Balance

O. B. Pratt, M.D., and H. O. Swartout, M.S., Los Angeles, Calif.

IT is now quite generally understood that maintenance of the acid-base balance is necessary for continued good health and that restoration of a disturbed balance is a valuable therapeutic measure in dealing with certain types of disease. A shift toward the acid side is sometimes desirable, an instance being the premedication which has been found useful in making urotropin active as a urinary antiseptic; but we much more frequently hear of alkalinizing the body; and in bringing about such a change diet is becoming increasingly useful. Many fruits and vegetables are rich in chemical compounds of the desired type, the most efficient being sodium or potassium salts of a few of the organic acids.

Biochemists have found that the salts that are effective alkalinizers undergo in nearly all cases as the first step in metabolism one of three processes: their organic acid radicals may be oxidized or otherwise decomposed, may unite with basic ions other than sodium or potassium to form insoluble or nonionized compounds, or may be joined with ammonia to form ammonium salts ready for excretion. In any of these events, sodium or potassium ions become available as active alkalinizing agents.

The alkalinizing power of citrates, readily administered in the form of citrus fruits or their juices, is well known. The case is not so clear for tartrates, which compose almost the entire quantity of the salts of organic acids in grapes, and which are not elsewhere found to any great extent.

The effects of tartrates, both upon lower animals and upon the human organism, have been extensively studied; but the results obtained are as yet inconclusive, especially with respect to their influence upon the acid-base balance. As long ago as 1914, Post,[1] having considered the reports of several men who had been testing the action of tartrates upon animals, and having had a question raised in his mind by comparing their adverse testimony with the claims of Fischer[2] that tartrates are beneficial in cases of edema and nephritis, gave Rochelle salts to a series of his patients, the quantities varying from one-half to three times the U. S. P. dose. His findings were: "(1) They give no evidence indicating that potassium and sodium tartrate in ordinary doses given by mouth in the human subject will cause albuminuria or cylindruria. (2) There is no evidence to show that tartrates ag-

*From the Research Laboratory, College of Medical Evangelists, White Memorial Hospital.
Received for publication, March 14, 1932.

gravate an existing nephritis. (3) The acidity of the urine, indicated by the hydrogen ion concentration, was as a rule less after the administration of tartrates."

The work and observations of Post should have gone a long way toward settling the tartrate question, but his conclusions have not been given the weight which we feel they deserve. In 1924, Rose[3] reviewed much of the literature on this subject and reported some of his own work. He noted that certain investigators had recovered from the urine quantities ranging from 8 per cent to 45 per cent of the tartrates administered to animals, that they had found albumin and casts in the urine of such animals, and that autopsy of the animals and microscopic examination of their kidneys had given evidence of injury to the tubules, especially in the convoluted portions. His own observations were confirmatory, his proof of kidney damage being that the composition of the blood of animals to which tartrates had been given showed the typical changes which are generally recognized as indicating decreased kidney function. He concluded: "Apparently tartrates are oxidized in the animal organism with great difficulty."

Simpson[4] gave tartrates to dogs, rabbits and cats, reporting the dosage calculated as tartaric acid. To dogs he gave by stomach tube from 0.35 gm. to 0.59 gm. per kg. of body weight and recovered from 17 per cent to 30 per cent from the urine. He gave subcutaneously from 0.14 gm. to 0.59 gm. per kg. and recovered from 47 per cent to 71 per cent. From rabbits he recovered a much smaller percentage of the dose introduced into the stomach; but he demonstrated that a rabbit's intestinal contents are capable of decomposing tartrates, if they are incubated together for twenty-four hours at body temperature. Cats were given from 0.54 gm. to 2.78 gm. per kg., and the amount recovered was from 44 per cent to 78 per cent. Animals showing a purgative effect from the tartrates excreted a smaller percentage of the dose through the kidneys, as might be expected. One of Simpson's conclusions was similar to that of Rose, but even stronger. He said: "It is, therefore, unlikely that tartaric acid is oxidized in the tissues."

Within the past few years many conflicting opinions about tartrates have been published. In 1926 a standard work[5] contained the following words: "The tartrates differ from the salts of most of the other vegetable acids in that they are not oxidized in the body but are eliminated unchanged through the kidney. It follows that they do not act as systemic alkalizers as do the citrates and acetates. The tartrates are also less readily absorbed from the intestines than the citrates, ..."

In 1927, Webster[6] published a small volume in which he gave a comprehensive review, with numerous abstracts, of the tartrate literature of our own and several other countries. Referring to an article by Curtis in the "Reference Handbook of Medicine," 1917 edition, and speaking of Rochelle salts, he said: "In non-purgative doses, especially if given well diluted, it is absorbed, changes to carbonate, then exerts, as efficiently as other salts, the specific action of alkaline potassium compounds." He also referred to the "British Pharmaceutical Codex," 1923 edition, page 60, and stated: "Tartaric acid is wholly or partly neutralized in the intestine, and a small portion is absorbed; . . . . The portion absorbed is for the most part oxidized and excreted in the urine as a carbonate, which renders the urine less acid: . . . ." These statements quoted by Webster agree in

general with Post's ideas, but they manifestly contradict the foregoing quotation from the "Dispensatory of the United States of America," with respect to the tartrates as alkalinizing agents.

The authors of three recent books that are now widely used as texts or references in pharmacology classes do not bring us much nearer to a solution of the tartrate problem. Solis-Cohen and Githens[7] say that both potassium bitartrate (the chief tartrate in grapes) and Rochelle salts are "alkalizers." McGuigan[8] says: "Tartrates are to some extent oxidized to carbon dioxide . . . ." According to Sollmann:[9] "The tartrates are not readily burned in the body, and therefore do not act as alkalis and diuretics as powerfully as do most other organic anions."

We should add a statement made by MacCallum[10] in discussing the edema believed to be due to certain poisons, for he mentions grape juice, which directly concerns our present subject: "Such substances as morphine, the juice of grapes and of various fruits, etc., are always spoken of in the textbooks as capable of producing such edema." We have not found grape juice so generally mentioned with disfavor as is here suggested, but MacCallum's statement at least serves to show its reputation among some men whose names and work are in high repute.

More recently, while our experiments were in progress, Pickens and Hetler[11] published their opinion that "Grape juice certainly does not function materially in decreasing the acidity of the urine." This conclusion was reached from tests made upon three normal women. It was not found that adding a pint or a quart of Welch's grape juice to a "basal" diet made any appreciable difference in the P_H value of the urine. Judging from their recorded data, the average "basal" P_H of their three subjects was about 6.

Recent contacts with dietitians and with physicians who are giving considerable attention to dietary problems have left us with the impression that not a few have the idea that tartrate-containing foods, which include grapes and grape products, are of doubtful value. There seems to be quite a widespread fear that these foods may on the one hand be harmful to the kidneys and on the other be of little or no use as alkalinizing agents. We do not claim to know all the reasons for these adverse opinions about tartrates, but we suspect that most of them are based upon the results of animal experimentation. In this connection we call attention to some pertinent observations of Post:[12] (1) Doses given to animals were larger in proportion to body weight than any ordinary dose would be for a human being. (2) Toxicity was greater when the tartrates were administered

TABLE I

TEST NO.	SUBJECT	WEIGHT OF GRAPES EATEN PER 24-HR. PERIOD	WEIGHT OF TARTRATES IN THESE GRAPES	VOLUME OF URINE PASSED DURING 24-HR. PERIOD	P_H OF THE URINE FOR 24-HR. PERIOD	AVERAGE P_H OF URINE FOR FIVE DAYS ON NORMAL DIET
I.	S	3000 G.	18.9 G.	1000 c.c.	7.0	6.4
II.	S	2600 G.	18.3 G.	1340 c.c.	7.0	6.4
II.	P	1850 G.	13.1 G.	1100 c.c.	6.6	6.1

subcutaneously than when they were introduced into the stomach. (3) Tartrates were more toxic to fasting animals than to those that were well fed, and the type of food affected the toxicity.

We do not deny the value of experiments made upon the lower animals, but we wish to emphasize the seemingly often forgotten truth that conclusions reached from such investigations are not always 100 per cent applicable to human beings.

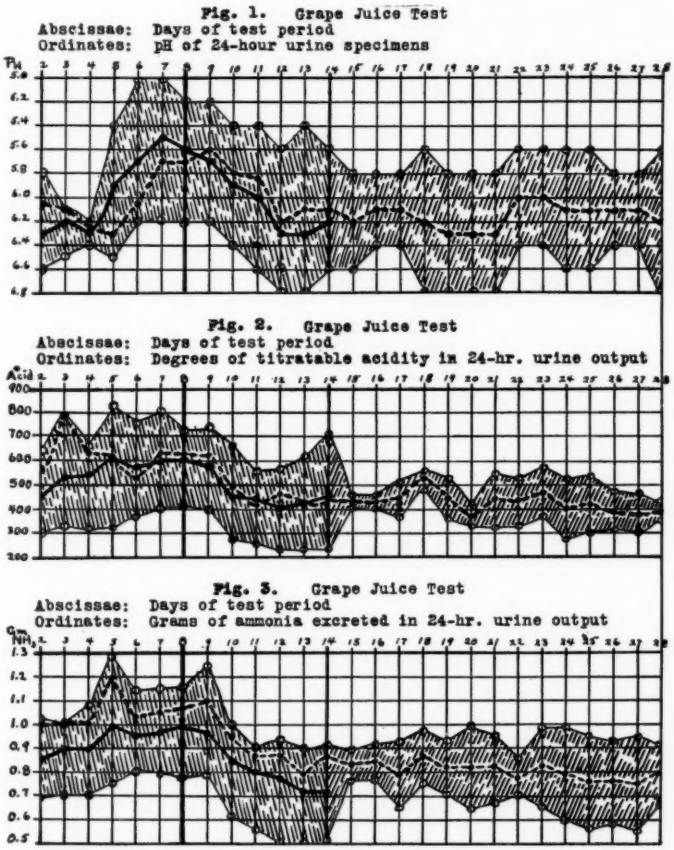


Fig. 1. Grape Juice Test
Abscissae: Days of test period
Ordinates: pH of 24-hour urine specimens
Fig. 2. Grape Juice Test
Abscissae: Days of test period
Ordinates: Degrees of titratable acidity in 24-hr. urine output
Fig. 3. Grape Juice Test
Abscissae: Days of test period
Ordinates: Grams of ammonia excreted in 24-hr. urine output

Figs. 1, 2, and 3.—A graphic record of the results of the test with grape juice on ten human subjects. In each figure the upper limit of the shaded area represents the most strongly acid condition found in any of the ten subjects, the lower limit the least acid, the solid curve the average of all ten subjects, and the broken curve the average of the two subjects whose test period was continued two weeks longer than the others. During the first seven days the subjects took an acid-forming control diet. The eighth day, marked by the heaviest vertical line, shows the beginning of the effect produced by adding grape juice to the diet. In Fig. 2 one "degree" of acidity represents the equivalent of 1 c.c. of N/10 acid.

Furthermore, different people vary so much in their reaction to any given set of conditions that we are constantly in danger of being too dogmatic in the generalizations that we draw from tests made upon but a few human subjects.

The work described below, a preliminary report[13] of which has already appeared, while not as extensive as we wish it might be, was more so than any of a similar sort that we have yet seen reported in the literature. We hope it will bring all who are interested a step nearer understanding the effects of tartrates on the human body. We have used a total of twenty subjects, some more than once.

TABLE II

SUBJECT	DAY OF TEST	VOLUME OF URINE PASSED	P_H OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
B	2	1200 c.c.	6.6	300°	0.75 gm.
B	3	1260	6.5	328	0.70
B	4	1310	6.4	314	0.71
B	5	1400	6.4	322	0.74
B	6	1330	6.0	372	0.81
B	7	1220	5.8	403	0.79
B	8	830	5.8	415	0.77
B	9	1280	5.8	397	0.79
B	10	810	6.4	275	0.62
B	11	1270	6.6	267	0.56
B	12	1050	6.6	231	0.50
B	13	1000	6.6	230	0.50
B	14	1000	6.6	240	0.51
D	2	1340	6.4	456	1.01
D	3	1450	6.2	594	0.98
D	4	1330	6.3	492	0.93
D	5	1120	6.1	616	1.10
D	6	1340	6.2	562	0.94
D	7	1140	6.2	570	1.07
D	8	870	5.6	626	1.13
D	9	1830	5.8	549	1.06
D	10	1380	6.2	455	0.87
D	11	1700	6.2	408	0.80
D	12	1100	6.4	352	0.77
D	13	1340	6.4	362	0.71
D	14	1050	6.4	294	0.57
J	2	700	6.4	462	0.70
J	3	650	6.1	520	0.75
J	4	690	6.2	669	0.94
J	5	1200	6.3	624	0.97
J	6	890	5.6	703	0.97
J	7	960	5.4	672	1.01
J	8	840	5.4	730	0.99
J	9	640	5.4	704	0.97
J	10	600	5.4	660	0.84
J	11	830	5.6	549	0.85
J	12	1470	6.4	353	0.80
J	13	1330	6.2	399	0.77
J	14	1760	6.4	458	0.79
L	5	700	5.4	420	
L	6	570	5.2	486	
L	7	700	5.4	476	
L	10	740	6.0	320	
L	12	940	6.6	284	
L	13	850	6.7	221	
M	2	1030	5.8	639	1.03
M	3	1300	6.1	793	1.01
M	4	1550	6.2	635	1.08
M	5	1640	6.1	771	1.29
M	6	1680	5.8	604	1.14
M	7	1730	5.4	744	1.15
M	8	900	5.2	702	1.16
M	9	1830	5.4	741	1.24
M	10	1790	5.2	537	1.00
M	11	1840	5.4	405	0.86
M	12	1680	5.6	504	0.94

TABLE II (CONTINUED)

SUBJECT	DAY OF TEST	VOLUME OF URINE PASSED	P_H OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
M	13	1500	5.4	510	0.89
M	14	2150	5.6	516	0.92
M	15	2060	5.8	453	0.89
M	16	2150	5.8	451	0.92
M	17	1650	5.8	511	0.93
M	18	2340	5.6	562	0.96
M	19	2110	5.8	527	0.93
M	20	2210	5.8	420	0.98
M	21	1930	5.8	559	0.95
M	22	1660	5.6	532	0.85
M	23	2230	5.6	580	0.98
M	24	2240	5.6	527	0.98
M	25	2350	5.6	521	0.95
M	26	2270	5.8	478	0.93
M	27	2500	5.8	475	0.94
M	28	2050	5.6	431	0.91
P	5	1550	5.4	837	
P	6	1800	5.4	684	
P	7	1500	5.4	620	
P	12	2050	6.6	565	
P	13	1150	6.0	628	
P	14	1620	6.2	713	
Q	5	1200	5.4	744	0.94
Q	6	900	5.0	764	0.98
Q	7	1000	5.0	812	0.94
Q	8	1030	5.4	576	0.87
Q	9	1700	6.2	536	0.85
Q	10	1500	6.4	468	0.81
Q	11	1750	6.4	547	0.83
Q	12	1800	6.4	468	0.84
Q	13	1800	6.4	540	0.80
R	2	1240	6.3	515	0.97
R	4	950	6.3	618	0.94
R	5	1770	6.5	561	1.10
R	6	930	6.3	465	0.94
R	7	1360	6.0	530	0.95
R	8	990	6.2	554	0.99
R	9	1180	5.8	496	0.95
R	10	1110	6.0	455	0.89
R	11	1060	6.3	445	0.90
R	12	1450	6.8	435	0.81
R	13	810	6.8	348	0.69
R	14	1120	6.6	381	0.82
R	15	1960	6.6	412	0.77
R	16	940	6.4	404	0.78
R	17	620	6.4	378	0.65
R	18	870	6.8	496	0.77
R	19	1395	6.8	363	0.71
R	20	890	6.8	329	0.65
R	21	1060	6.8	329	0.68
R	22	1040	6.4	339	0.71
R	23	1250	6.4	388	0.66
R	24	910	6.6	273	0.60
R	25	900	6.6	297	0.56
R	26	880	6.4	308	0.58
R	27	820	6.4	301	0.55
R	28	630	6.8	360	0.66

TABLE II (CONTINUED)

SUBJECT	DAY OF TEST	VOLUME OF URINE PASSED	P_H OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
S	5	1460	5.4	681	
S	6	1350	5.4	630	
S	7	960	4.8	672	
S	12	1400	6.6	427	
S	13	1140	6.6	350	
S	14	1160	6.0	614	
T	2	930	6.2	344	0.69
T	3	940	6.2	413	0.96
T	4	930	6.3	521	0.74
T	5	810	6.2	510	0.78
T	6	640	6.0	448	0.87
T	7	1070	5.6	482	0.89
T	8	870	5.4	618	1.04
T	9	950	5.2	636	0.92
T	10	880	5.6	493	0.83
T	11	1040	5.8	478	0.78
T	12	1110	5.6	433	0.77
T	13	960	5.8	374	0.70
T	14	1130	5.6	416	0.71

They were all in good health and ranged in age from the early twenties to the early forties.

In our first two tests the urinary P_H of subjects P and S was determined for five consecutive days while they were on their normal lacto-ovo-vegetarian diet. On the sixth and seventh days they ate nothing but grapes. Twenty-four-hour urine specimens were collected the seventh day. Tests were made as indicated in Table I, tartrates being calculated as potassium acid tartrate.

In the third test, subject S ate 450 gm. of Thompson seedless raisins daily for seven days, together with 50 gm. of nut margarine, three eggs, and enough bread and other cereal products to make a total diet of approximately 3000 calories a day. Water was the only liquid taken. The degree of urinary acidity that might be expected from such a diet, if raisins were left out, can be judged from the effect of our control diet for the fourth test, which was quite similar to the third test diet minus the raisins. This control diet is described in the second paragraph below. It gave subject S a urinary P_H of 5.2. The quantities of urine passed for the last three days of the raisin diet period were 1200 c.c., 1280 c.c. and 1170 c.c., respectively. The urinary P_H on each of these days was 6.6.

These first three tests were made chiefly with the idea of getting data that would help us decide whether or not we should study our subject more extensively. The uniformity with which the results pointed to the alkalinizing nature of grape tartrates led us to go on. We made no more tests with raisins, however, for Blatherwick's[14] report about their power to reduce urinary acidity seems to be quite generally accepted, and one of the lay publications[15] recently referred to some work done in the Fruit Products Laboratory of the University of California, showing the value of raisins in overcoming acidosis.

Subjects L, P, Q, and S were used in the fourth test. Each subject at first took a control diet for one week, composed of three or four eggs a day, 30 to 50 gm.

of butter or nut margarine, 250 to 400 gm. of bread or its equivalent in other cereal foods, as much milk as desired, and occasionally small quantities of walnuts, cheese, canned green corn, or some other acid-forming or but slightly alkalinizing vegetable. During the second week the same general dietary program was continued, except for the elimination, or the reduction in quantity, of milk, which is approximately neutral with respect to the formation of acid or base in the body;

Fig. 4. Grape Test
Abscissae: Days of test period
Ordinates: pH of 24-hour urine specimens

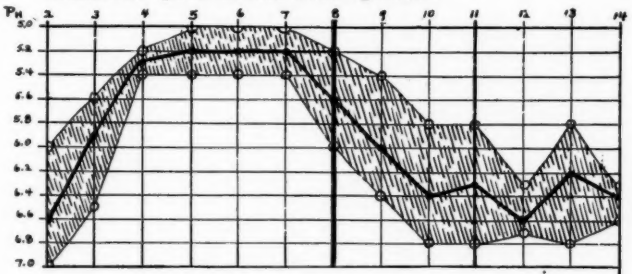


Fig. 5. Grape Test
Abscissae: Days of test period
Ordinates: Degrees of acidity in 24-hr. urine output

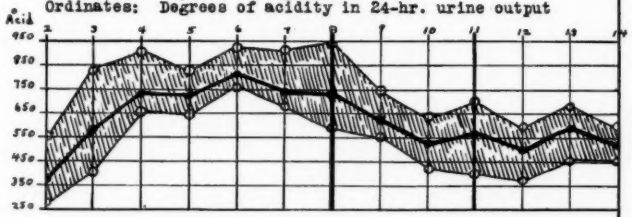
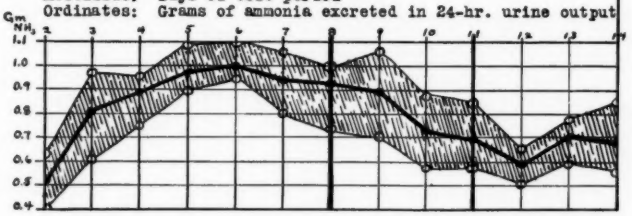


Fig. 6. Grape Test
Abscissae: Days of test period
Ordinates: Grams of ammonia excreted in 24-hr. urine output



Figs. 4, 5, and 6.—A graphic record of the results of the test with grapes on six human subjects. In each figure the upper limit of the shaded area represents the most strongly acid condition found in any of the six subjects, the lower limit the least acid, and the solid curve the average of all six subjects. During the first seven days the subjects took an acid-forming control diet. The eighth day, marked by the heaviest vertical line, shows the beginning of the effect produced by adding fresh grapes to the diet. Beginning with the eleventh day, marked by the medium heavy vertical line, the grapes chosen had a somewhat lower tartrate content than those previously eaten. In Fig. 5, one "degree" of acidity represents the equivalent of 1 c.c. of N/10 acid.

and each subject took one liter of grape juice a day. We used a blend of the juices of Concord and Worden grapes, with a tartrate content of 0.93 per cent.

While Test IV was in progress it occurred to us that a test of urinary P_H alone would not be so conclusive as a combined check of P_H, titratable acidity and ammonia output, since all three have to do with the acid-base balance. We were able to obtain data on P_H and titratable acidity from all four subjects and on ammonia output also from subject Q.

TABLE III

SUBJECT	DAY OF TEST	VOLUME OF URINE PASSED	P_H OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
C	2	1600 c.c.	6.9	336°	0.54 gm.
C	3	1750	6.5	413	0.80
C	4	1020	5.2	765	0.95
C	5	1320	5.4	686	0.92
C	6	920	5.4	773	0.94
C	7	1500	5.2	915	0.99
C	8	1540	5.2	678	0.99
C	9	1320	6.0	726	0.89
C	10	1640	6.8	426	0.70
C	11	1540	6.8	400	0.63
C	12	1430	6.7	372	0.56
C	13	1360	6.4	558	0.72
C	14	1660	6.6	515	0.65
E	2	930	6.7	279	0.40
E	3	760	5.6	418	0.62
E	4	950	5.2	665	0.90
E	5	840	5.0	697	0.97
E	6	1160	5.2	801	0.94
E	7	920	5.2	672	0.88
E	8	1080	5.8	583	0.73
E	9	1100	6.2	572	0.70
E	10	1120	6.8	470	0.58
E	11	1080	6.6	454	0.58
E	12	825	6.7	422	0.51
E	13	1460	6.8	453	0.59
E	14	1460	6.6	453	0.60
F	2	650	6.3	312	0.45
F	3	1470	5.8	544	0.84
F	4	1100	5.4	660	0.85
F	5	800	5.2	640	0.89
F	6	1200	5.0	800	0.98
F	7	1380	5.0	717	1.01
F	8	900	5.6	748	0.99
F	9	1760	5.8	634	1.01
F	10	1540	6.0	547	0.88
F	11	1120	6.0	526	0.63
F	12	970	6.3	602	0.63
F	13	980	6.4	672	0.73
F	14	1600	6.3	549	0.73
G	2	1620	6.5	444	0.60
G	3	1100	5.6	825	0.97
G	4	1300	5.4	910	0.94
G	5	1100	5.4	825	1.09
G	6	1170	5.2	936	1.03
G	7	1120	5.4	672	0.89
G	8	1180	6.0	732	0.91
G	9	1600	6.4	640	0.95
G	10	900	6.2	567	0.67
G	11	1100	6.2	704	0.75
G	12	820	6.3	549	0.60
G	13	1160	5.8	678	0.71
G	14	1620	6.5	535	0.68
H	2	830	6.0	556	0.58
H	3	750	5.6	645	0.68
H	4	680	5.2	680	0.75
H	5	1000	5.0	837	0.98

TABLE III (CONTINUED)

SUBJECT	DAY OF TEST	VOLUME OF URINE PASSED	P_H OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
H	6	820	5.0	800	0.97
H	7	780	5.2	749	0.80
H	8	670	5.2	943	0.99
H	9	700	5.4	560	0.70
H	10	780	5.8	640	0.65
H	11	780	5.8	632	0.72
H	12	1020	6.6	587	0.58
H	13	900	5.8	603	0.67
H	14	720	6.3	497	0.56
K	2	1300	7.0	312	0.64
K	3	1200	6.2	612	0.92
K	4	620	5.2	713	0.94
K	5	950	5.4	712	0.97
K	6	820	5.4	763	1.11
K	7	1460	5.4	730	1.05
K	8	1730	5.6	717	0.98
K	9	900	6.0	747	1.07
K	10	1000	6.8	530	0.82
K	11	960	6.6	672	0.84
K	12	1000	6.6	460	0.65
K	13	1120	6.2	547	0.78
K	14	1560	6.3	593	0.85

In completing Test IV we used six more subjects: B, D, J, M, R, and T. Their preliminary diet was similar to that already described, though not so rigidly controlled; and their urine was given the triple test. M and R were asked to extend their test period until they had taken grape juice for three weeks, so that we might get a better idea of the permanence of the changes that occurred. Data obtained from the ten subjects are given in Table II, and the significant results are graphically illustrated in Figs. 1, 2, and 3.

Subjects C, E, F, G, H, and K were used in a test similar to Test IV in every respect except that 1 kg. of fresh grapes replaced each liter of grape juice. Several different varieties of grapes were used, a comparatively watery variety, low in tartrate content, forming the bulk of the grape ration the last four days of the test period. Our purpose was to see if a continuance of the grape diet, but a decrease in the tartrates ingested, would be marked by any significant change in the data obtained by our triple check on the urine passed by the six subjects. The data for this, Test V, are given in Table III, and the results are graphically shown in Figs. 4, 5, and 6.

In Test VI subjects I, N, and S took a preliminary diet similar to that used in Tests IV and V, taking also Rochelle salts (COOK-CHOH-CHOH-COONa) with every meal during the second week. The results are given in Table IV. Table V gives the data from Test VII, made on subjects A and O. Test VII was parallel to Test VI, except that potassium bitartrate (COOK-CHOH-CHOH-COOH) was used instead of Rochelle salts.

A study of Tables I to V and Figs. 1 to 6 shows that tartrates, whether given as normal or acid salts or in the form of grapes or grape products, proved to be alkalinizing in every case, though different subjects were affected to different de-

TABLE IV

SUBJECT	DAY OF TEST	DAY'S DOSE OF TARTRATES	VOLUME OF URINE PASSED	P_H OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
I	2	0 gm.	940 c.c.	6.2	583°	0.87 gm.
I	3	0	720	5.6	590	0.86
I	4	0	1300	6.2	715	1.21
I	5	0	880	5.6	678	1.12
I	6	0	1300	5.8	637	1.10
I	7	0	1070	5.2	663	1.12
I	8	12	990	5.2	614	1.04
I	9	15	1240	6.2	496	0.95
I	10	15	930	6.8	307	0.60
I	11	18	1250	6.6	375	0.63
I	12	18	1170	6.8	304	0.79
I	13	20	1200	7.0	192	0.65
I	14	20	1170	6.8	281	0.54
N	2	0	1110	6.2	522	1.01
N	3	0	950	6.0	665	1.08
N	4	0	1060	5.6	668	1.21
N	5	0	875	5.8	560	0.99
N	6	0	960	5.4	595	1.30
N	7	0	1000	5.2	555	1.18
N	8	12	1510	5.2	529	1.18
N	9	15	1170	6.4	410	0.95
N	10	15	1040	6.8	270	0.65
N	11	18	1020	7.2	173	0.57
N	12	18	1020	6.6	306	0.63
N	13	20	950	7.0	262	0.53
N	14	20	1010	6.8	212	0.60
S	4	0	1620	5.2	729	0.74
S	5	0	1530	5.4	658	0.78
S	6	0	1460	5.4	657	0.75
S	7	0	1250	5.6	550	0.70
S	8	12	1850	6.4	516	0.56
S	9	12	1600	6.6	514	0.44
S	10	12	1700	6.6	510	0.49
S	11	12	1470	6.4	547	0.58

grees. Rochelle salts showed the greatest alkalinizing power, and potassium bitartrate the least; but when one thinks of the chemical formulas of these two salts this difference is not hard to understand, the molecule of potassium bitartrate containing but one alkali metal atom, while the molecule of Rochelle salts contains two. With respect to the negative results obtained by Pickens and Hetler with grape juice, we make no comment except to say that we agree with a statement in the *Journal of the American Medical Association*,[16] which, referring to their work, said: "One puzzling aspect of the behavior of the unfermented, sweetened grape juice used in these studies is the fact that raisins are known to decrease the acidity of the urine."

Chemical and microscopic tests on all urine specimens failed to show the presence of either tartrates, albumin, or casts in any case. None of the subjects complained of any ill-feeling. Grapes and grape juice caused a mild catharsis at first in a few cases, but this disappeared within two or three days. Neither Rochelle salts nor potassium bitartrate produced catharsis. Data in the tables show no noticeable diuresis as a result of taking tartrates along with a normal

TABLE V

SUBJECT	DAY OF TEST	DAY'S DOSE OF TARTRATES	VOLUME OF URINE PASSED	P_H OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
A	2	0 gm.	1050 c.c.	5.4	493°	0.65 gm.
A	3	0	720	4.8	562	0.62
A	4	0	1000	5.0	750	0.73
A	5	0	835	4.8	726	0.78
A	6	0	670	5.2	670	0.75
A	7	0	850	4.8	655	0.88
A	8	3	890	5.0	632	0.98
A	9	6	860	5.0	636	0.91
A	10	6	740	5.2	540	0.75
A	11	9	960	5.2	643	0.78
A	12	9	750	5.4	373	0.62
A	13	12	840	5.4	398	0.66
A	14	12	750	5.6	330	0.50
O	2	0	1430	5.8	611	1.01
O	3	0	910	6.2	692	0.99
O	4	0	880	5.4	581	0.81
O	5	0	845	5.4	668	1.00
O	6	0	930	5.6	632	1.12
O	7	0	890	5.4	534	1.01
O	8	3	1550	5.4	620	1.08
O	9	6	1020	5.2	663	1.16
O	10	6	1120	5.8	560	1.03
O	11	9	1410	5.4	522	0.99
O	12	9	940	5.4	536	0.87
O	13	12	1550	5.4	527	0.84
O	14	12	1570	5.8	465	0.85

quantity of food, expect perhaps a mild degree in the case of subjects M and O. We conclude from our work, therefore, that grapes, raisins, and grape juice should be considered as harmless and effective additions to the list of foods that may be used in making up an alkalinizing diet.

SUMMARY

1. A series of seven tests was made on a total of twenty healthy human subjects, to determine the effect of adding grapes, raisins, grape juice, Rochelle salts, or potassium bitartrate to an otherwise acid-forming diet.

2. All these substances were definitely alkalinizing, though potassium bitartrate was less so than the others.

3. No proof of kidney damage or other injury could be found in any case.

4. Our work, therefore, indicates that grapes, raisins, and grape juice are harmless and effective alkalinizing foods.

REFERENCES

1. Post, W. E.: The Effect of Tartrates on the Human Kidney, J. A. M. A. **62**: 592, 1914.
2. Fischer, M. H.: Oedema, New York, 1910, John Wiley & Sons, p. 116, etc.; Nephritis, New York, 1912, John Wiley & Sons, p. 135.
3. Rose, W. C.: The Nephropathic Action of the Dicarboxylic Acids and Their Derivatives, I., J. Pharmacol. & Exper. Therap. **24**: 123, 1924.
4. Simpson, G. E.: Urinary Excretion of Tartrates Following Administration to Animals, J. Pharmacol. & Exper. Therap. **25**: 459, 1925.
5. Wood, H. C., LaWall, C. H., and Others: The Dispensatory of the United States of America, ed. 21, Philadelphia, 1926, J. B. Lippincott Company, p. 878.

6. Webster, R. W.: Potassium and Tartrates, Chicago, 1927, The Commonwealth Press, pp. 111-156.
7. Solis-Cohen, S., and Githens, T. S.: Pharmacotherapeutics, Materia Medica and Drug Action, New York, 1928, D. Appleton and Company, pp. 1175 and 1176.
8. McGuigan, H. A.: A Text-Book of Pharmacology and Therapeutics, Philadelphia, 1928, W. B. Saunders Company, p. 282.
9. Sollmann, T.: A Manual of Pharmacology, Philadelphia, 1928, W. B. Saunders Company, p. 824.
10. MacCallum, W. G.: A Text-Book of Pathology, Philadelphia, 1928, W. B. Saunders Company, p. 47.
11. Pickens, L. M., and Hetler, R. A.: The Effect of Grape Juice on Nitrogen Retention and Urinary Acidity, J. Home Econ. **22**: 44, 1930.
12. Post, W. E.: The Effect of Tartrates on the Human Kidney, J. A. M. A. **62**: 592, 1914.
13. Pratt, O. B., and Swartout, H. O.: Effect and Mode of Action of Tartrates in the Human Body, Proc. Soc. Exper. Biol. & Med. **27**: 1037-1038, 1930.
14. Blatherwick, N. R.: The Specific Rôle of Foods in Relation to the Composition of the Urine, Arch. Int. Med. **14**: 409, 1914.
15. Calif. Cultivator **76**: 535, 1931. Confirmed by personal communication from the Laboratory, Oct. 29, 1931.
16. Current Comment, Grape Juice and the Acidity of the Urine, J. A. M. A. **94**: 1070, 1930.

TARTRATE METABOLISM*

II. The Mode of Action of Ingested Tartrates

O. B. Pratt, M.D., and H. O. Swartout, M.S., Los Angeles, Calif.

IN CONNECTION with work reported in the first part of this paper[1] under the subtitle "Grape Tartrates and the Acid-Base Balance" there were developments which led us to study further into the way ingested tartrates are handled in the human body. We found that grapes, raisins, grape juice, and the alkali salts of tartaric acid decreased the acidity of the urine of all the subjects used in our experiments. This finding, though it differed from that of Pickens and Hetler[2] about grape juice, was in harmony with the results obtained by Post[3] with Rochelle salts and with what was long ago found to be true concerning raisins by Blatherwick[4] and recently by workers in the Fruit Products Laboratory of the University of California.[5] But, because of the uncertainty regarding the method by which tartrates produce their observed effect, an uncertainty largely due to the contradictory nature of statements found in periodicals and textbooks, we have given particular attention to three questions: (1) Are tartrates absorbed to an appreciable extent from the human intestine? (2) If absorbed, are they oxidized in the tissues? (3) If not absorbed and oxidized, how can they be alkalinizing?

If tartrates were found in the urine after ingestion by human subjects, it would be evidence of their absorption through the wall of the gastrointestinal tract and into the blood as preliminary steps to their excretion by the kidneys; but we did not find them present in such cases. It is possible that traces were present, for we could not devise a practical procedure that would detect tartrates in a solution more dilute than one part in two thousand. It is also possible that the urinary excretion of tartrates might have occurred if the human dosage had been proportionally as large as some of the doses used in animal experimentation. The largest dose we

*From the Research Laboratory, College of Medical Evangelists, White Memorial Hospital. Received for publication, March 14, 1932.

used was 20 gm. a day, which would surely seldom be exceeded in any diet or in the treatment of the sick. We were not trying to determine how much tartrate would need to be ingested to injure the kidneys, but rather to test the effect of quantities that might reasonably be prescribed in medical practice, either as medication or in the diet.

Tartrates, especially Rochelle salts, are pharmacologically classed both as saline cathartics and as diuretics. A cathartic action argues against much absorption, for the effectiveness of saline cathartics depends partly upon difficulty of absorption. On the other hand, it is not easy to see how an ingested salt can act as a diuretic unless it is absorbed from the intestine and carried by the blood to the kidneys. There is, however, another possible explanation for tartrate diuresis, as will be seen later in this discussion.

The alkalinizing effect of tartrates, which our former tests tended to prove, would be strong evidence of their oxidation in the tissues if their absorption from the intestine were known to occur; but the results of our recent experiments have convinced us that neither such absorption nor such oxidation are necessary in order to produce this effect. While we have, therefore, not found the final answer to the first two of the three questions stated above, we believe that they do not need to be answered in the affirmative.

The clue which we have followed in trying to answer the third question came from Simpson's[6] statement that a rabbit's intestinal contents can decompose tartrate ions in twenty-four hours and that a bouillon culture of Escherichia communior can do so in twelve days. In view of the important possibilities which such a finding opens up, it is surprising that no record of further work along this line has appeared in the literature since his report.

Three hours after a meal we gave one of our subjects 10 gm. of magnesium sulphate. A fluid stool specimen was obtained. Three flasks were prepared, each containing 10 c.c. of the stool and 15 c.c. of water. In the first was placed 0.1 gm. of Rochelle salts (COOK-CHOH-CHOH-COONa), in the second 0.1 gm. of potassium acid tartrate (COOK-CHOH-CHOH-COOH), and the third 0.1 gm. of tartaric acid (COOH-CHOH-CHOH-COOH). All three flasks were incubated at 37° C. for twenty-four hours and tests made for the presence and quantity of tartrates. Results are shown in Table I.

TABLE I

FLASK 1	FLASK 2	FLASK 3
No tartrate present	0.04 gm. tartrate	0.07 gm. tartrate

A 5 gm. portion of a normal stool specimen from the second subject was emulsified with 25 c.c. of water and 0.3 gm. of Rochelle salts. After twenty-four hours' incubation at 37° C. no tartrate could be recovered.

Normal stool specimens were obtained from the third and the fourth subjects. Three series of five flasks each were prepared, each flask containing 25 c.c. of water and 0.2 gm. of Rochelle salts. In each flask of the first series we placed 2 gm. of the third subject's stool, in each flask of the second series 5 gm. of the third subject's stool, and in each flask of the third series 5 gm. of the fourth subject's stool. The flasks were incubated for different lengths of time. Our plan was to get some idea of the rate at which different quantities of feces would decompose tartrate ions. In-

cubation periods and tartrate losses in approximate percentages are given in Table II.

TABLE II

SERIES	FLASK 1 2 HOURS	FLASK 2 3 HOURS	FLASK 3 4 HOURS	FLASK 4 6 HOURS	FLASK 5 24 HOURS
1	10% loss	20% loss	35% loss	50% loss	100% loss
2	40% loss	50% loss	60% loss	80% loss	100% loss
3	50% loss	70% loss	90% loss	100% loss	100% loss

We followed this line of investigation further, using stool specimens sent to our clinical laboratory for analysis. In each case approximately 5 gm. of the stool was put into a flask with 50 c.c. of water and 0.2 gm. of the tartrate being tested at the time. We prepared 60 flasks, using Rochelle salts, and 50, using potassium acid tartrate. All were incubated at 37° C. for twenty-four hours and the contents of the flasks tested for tartrates. One hundred and seven of the 110 flasks showed no tartrates at all. Two showed heavy traces. One showed about 0.1 gm. For the two flasks we had purposely chosen stool specimens that were hard and dry and about a week old when tested. That used in the one flask consisted of a mixture of unmasticated, sliced, raw onions and yellowish mucus, passed by a toothless old lady who had developed a diarrhea after indulging a perverted appetite a bit too far.

The above findings show that the normal human intestinal contents are capable of decomposing tartrates at a rate which would insure the destruction of all the tartrate ions in a U. S. P. dose of Rochelle salts or potassium acid tartrate in much less than the time food normally remains in the absorbing part of the intestine. Catharsis might hasten the passage of food along the digestive tract enough to cause this decomposition to be incomplete, but in any case it should take place to a considerable extent.

Table I shows that the less acid the tartrates are the more easily they are broken down. Normally, the intestinal contents possess sufficient alkalinity to neutralize all the potassium acid tartrate that would ordinarily be ingested; and tartaric acid, as such, is seldom taken into the human stomach in appreciable amounts. The normal physiologic condition of the intestine, therefore, would favor the process of tartrate decomposition.

Further tests were made to determine what constituent or constituents of the feces might be responsible for the observed decomposition. These tests are described below in sufficient detail to make the purpose and procedures clear.

Thirty cubic centimeters of human bile was collected by means of a duodenal tube. Ten cubic centimeters was placed in each of three flasks, along with 0.2 gm. of Rochelle salts and 40 c.c. of water. The three flasks were incubated for five days without loss of tartrates.

Into each of five flasks we placed 1 gm. of pancreatin, 0.2 gm. of Rochelle salts and 50 c.c. of water. These flasks were also incubated for five days. No loss of tartrates could be detected in any of the flasks.

Ninety flasks were prepared and divided into three lots of thirty each, each flask containing 50 c.c. of plain nutrient broth. One lot was inoculated with Escherichia coli, one with Escherichia communior, and one with Escherichia acidi-lactici. All were incubated for twenty-four hours at 37° C. Into each flask we then put 0.2 gm. of Rochelle salts and incubated all of them for twenty-four hours more.

Those inoculated with E. coli and E. communior showed complete decomposition of tartrates. The first few of the flasks tested that had been inoculated with E. acidi-lactici showed heavy traces of tartrates still undecomposed. After another twenty-four hours of incubation of the remainder of the flasks, the decomposition was found to be complete.

We next took 50 gm. portions of seventeen different stools that came into the clinical laboratory. Each portion was emulsified with ten times its weight of water and filtered through a Berkefeld filter. Enough filtrate was obtained to give 50 c.c. for each of 78 flasks. The first ten were inoculated with E. coli, the second ten with E. communior and the third ten with E. acidi-lactici. All the flasks were incubated at 37° C. for twenty-four hours, 0.2 gm. of Rochelle salts was added to each flask, and the incubation continued for forty-eight hours more. No tartrates could be recovered from any of the thirty inoculated flasks, but there seemed to be no loss of tartrates in the remaining 48 uninoculated flasks.

The four tests just described indicate that the decomposition of tartrates by feces is not due to chemicals or enzymes per se, but rather to the action of living bacteria, particularly to that of the two common varieties of colon bacillus. We made further tests, however, to see if an alkalinizing effect could be produced by such bacterial action outside the human body.

Twelve pour plates were prepared, using plain nutrient agar to which a little brom thymol blue and 1 per cent of Rochelle salts had been added and the P_H adjusted to 6.4, giving a greenish-yellow tint. Four of the plates were streaked with E. coli, four with E. communior, and four were left sterile for controls. All were incubated at 37° C. After twenty-four hours there was a distinct blue halo about the colonies of bacteria on each of the inoculated plates, showing that alkali had appeared as a result of the bacterial action. After forty-eight hours the blue color had spread throughout the agar. The color in the sterile plates had changed very little, at most enough to indicate a shift of P_H from 6.4 to 6.6.

Fifty cubic centimeters of plain nutrient broth was put into each of twelve flasks. All were inoculated with E. coli after the P_H had been adjusted to 7.6. Three-tenths cubic centimeter of alcoholic phenolphthalein solution was then dropped into each flask and 0.2 gm. of Rochelle salts was put into each of six of the flasks. All were incubated in the usual way. Within forty-eight hours a pink tinge appeared in four of the flasks to which Rochelle salts had been added, and after twenty-four hours more each of these six flasks showed a distinct light reddish-brown color. The color of the broth in the six flasks to which no Rochelle salts had been added showed no such change, though the broth became cloudy from the growth of bacteria.

We cannot prove the nature of the tartrate decomposition which occurred in these tests. It is likely, however, that carbon dioxide and water were formed. If so, whenever Rochelle salts was the tartrate used, both sodium and potassium bicarbonates would be among the decomposition products. These could produce the observed alkalinizing effect. Furthermore, if such a series of chemical reactions occurred in the human intestine, these bicarbonates would readily be absorbed and could easily cause the mild diuresis sometimes noticed after the ingestion of tartrates.

As a test for bicarbonates, we added a few drops of strong hydrochloric acid to

each of the twelve flasks used in the last test mentioned above, after the seventy-two hours' incubation. A few very small bubbles of gas arose from each, as near as we could estimate about three or four times as many from the flasks to which Rochelle salts had been added as from the control flasks, but we could not call what we saw an unmistakable effervescence. We have evidence, therefore, but it is not conclusive enough to amount to proof, that bicarbonates were formed as a result of bacterial action on the Rochelle salts in these test flasks.

Our conclusion from the two tests last described is that the common fecal bacteria are in the course of their growth able to decompose tartrates, and thereby increase the alkalinity of the medium in which this growth and decomposition are taking place. If, as we feel justified in believing, the acid radicals of ingested alkali tartrates are largely or wholly broken down in the human intestine by bacterial action, we have a logical explanation of all our findings. First, the decomposition of tartrate ions would set free easily absorbable sodium and potassium ions, which could produce the observed alkalinizing effect. Second, if the tartrate ions were decomposed before absorption, we should expect to see exactly what we did see, no signs of kidney damage and no tartrates in the urine. Third, the occasional mild diuresis observed could have been due to the decomposition products of tartrates, probably bicarbonates, rather than to the tartrates themselves.

SUMMARY

1. In our previous work we found ingested tartrates to be alkalinizing in the human body, but the method by which this effect is produced was not settled.

2. We have recently given attention to three questions: (1) Are tartrates absorbed from the human intestine? (2) If absorbed, are they oxidized in the tissues? (3) If not absorbed and oxidized, how can they be alkalinizing?

3. If tartrates are absorbed, they are oxidized in the tissues, for the human kidney does not excrete more than traces of tartrates, if any at all, after their ingestion.

4. Tartrates are decomposed by feces and by the common fecal bacteria, but not by bile, pancreatin or Berkefeld filtrates of feces. The ease and rapidity with which this decomposition takes place shows that at least the major portion of ingested tartrates can normally be decomposed in the lumen of the intestine.

5. In the process of decomposition of the alkali salts of tartaric acid by cultures of fecal organisms, the medium in which the decomposition takes place is made more alkaline. It is, therefore, not necessary for tartrates to be absorbed from the intestine and oxidized in the tissues in order to exert their characteristic influence upon the human body. The absorption of the decomposition products can account for the alkalinizing effect which was uniformly observed, as well as the mild diuresis which was occasionally noticed.

REFERENCES

1. Pratt, O. B., and Swartout, H. O.: Tartrate Metabolism. I. Grape Tartrates and the Acid-Base Balance, J. Lab. & Clin. Med. **18**: 354, 1933.
2. Pickens, L. M., and Hetler, R. A.: The Effect of Grape Juice on Nitrogen Retention and Urinary Acidity, J. Home Econ. **22**: 44, 1930.
3. Post, W. E.: The Effect of Tartrates on the Human Kidney, J. A. M. A. **62**: 592, 1914.
4. Blatherwick, N. R.: The Specific Rôle of Foods in Relation to the Composition of the Urine, Arch. Int. Med. **14**: 409, 1914.
5. Personal Communication, Oct. 29, 1931.
6. Simpson, G. E.: Urinary Excretion of Tartrates Following Administration to Animals, J. Pharmacol. & Exper. Therap. **25**: 459, 1925.

A STUDY OF THE OCCURRENCE OF UNFERTILIZED ASCARIS EGGS*

A. E. KELLER, M.D., NASHVILLE, TENN.

THE diagnosis of the intestinal helminths which are commonly found in man can be made readily by any one of the numerous methods of fecal examination. Most of the eggs which are found in the feces are characteristic for each worm. In the case of Ascaris lumbricoides, however, both fertilized and unfertilized eggs may be found. This fact introduces a possible source of error in the diagnosis of this particular parasite. There is not very much information available concerning the frequency with which fertilized and unfertilized Ascaris eggs occur in cases of ascariasis.

Fertilized eggs are very characteristic, having a thick, clear, inner shell covered by an irregular, warty, albuminous coat which is stained yellow or brown in the intestine. They are usually spherical or slightly oval in shape and measure from 45 to 75 micra in length by 35 to 50 micra in width. Unfertilized eggs are much longer, narrower, more elliptical in shape. They measure from 70 to 88 micra in length by 38 to 50 micra in width. The inner structure is unorganized and frequently contains amorphous granular material or it may be vacuolated. The albuminous coat may be absent from both the fertilized and unfertilized eggs. The unfertilized eggs are usually bile stained but are more likely to be colorless, gray, or unstained than are fertilized eggs. Fig. 1 shows both fertilized and unfertilized Ascaris eggs. These illustrations were furnished by and are used with the permission of Doctors G. F. Otto of the Department of Helminthology of the Johns Hopkins School of Hygiene and Public Health and John E. Stumber of the Rockefeller Institute for Medical Research.

This study was undertaken to ascertain the following points: (a) the frequency with which unfertilized Ascaris eggs occur alone in fecal specimens; (b) the frequency with which fertilized Ascaris eggs occur alone; (c) the frequency with which both kinds of Ascaris eggs appear; (d) the relative proportion of fertilized and unfertilized eggs in cases where both kinds were present in the specimen.

In making this differential Ascaris egg-count study a series of 1,103 specimens of feces which contained Ascaris eggs were used. A single egg-count on each specimen was made, using the Stoll egg-counting method. By using this method the intensity of infestation was determined and the approximate percentage of fertilized eggs in each specimen was recorded according to Table I which shows the relative proportion of fertilized eggs estimated to be present in each specimen.

Group 1 contains no fertilized eggs or unfertilized eggs alone. The percentage of fertilized eggs increases until group 8 is reached in which 100 per cent of the eggs are fertilized.

*From the Department of Preventive Medicine and Public Health of Vanderbilt University School of Medicine.
Received for publication, March 9, 1932.

All of the egg-counts were done by two technicians who were well trained both in the diagnosis of these helminths and in the use of the Stoll egg-counting technic. The specimens were obtained from school children in the eastern and middle sections of Tennessee. The average intensity of infestation for the entire series was 108,300 Ascaris eggs per gram of feces.

Table II shows the frequency of the two kinds of Ascaris eggs in this series. In 26.2 per cent of the specimens unfertilized eggs alone were present while in 32.2 per cent of the specimens all of the eggs were fertilized. In 41.6 per cent

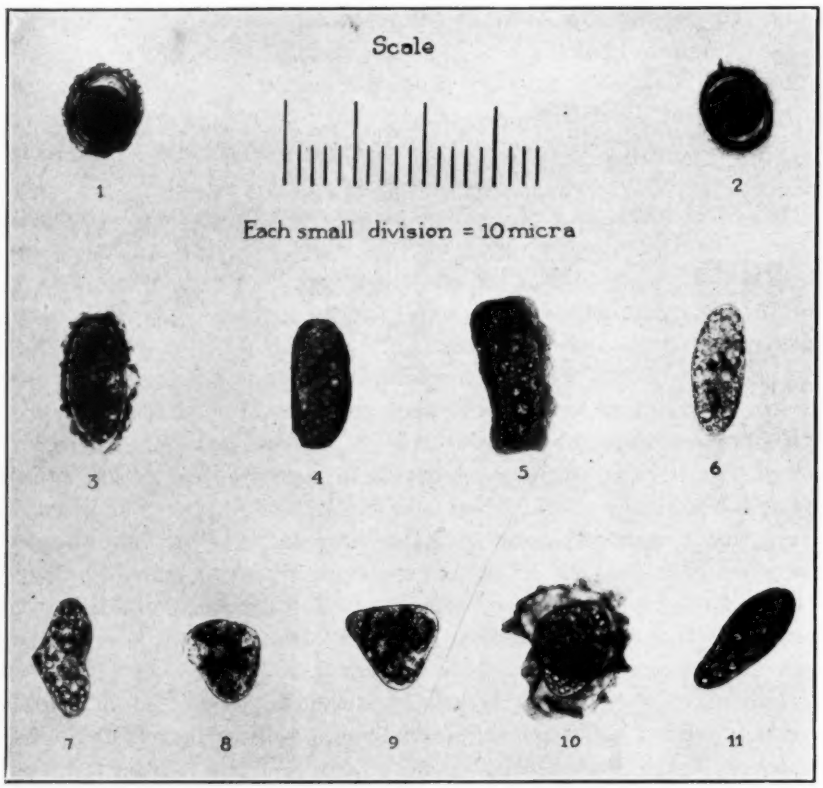


Fig. 1.—Fertilized and unfertilized Ascaris eggs. No. 1 and 2 typical fertilized eggs with albuminous capsule. Nos. 3, 4, 5, and 10 unfertilized eggs with albuminous capsule. Nos. 6, 7, 8, 9, and 11 unfertilized eggs without albuminous capsule.

both fertilized and unfertilized eggs were noted. It can be seen that unfertilized eggs alone were found almost as frequently as fertilized eggs.

In determining the relationship between the intensity of infestation and the percentage of fertilized eggs, the following intensity grouping was used.

By using this classification it is possible to determine approximately the total number of worms present as it is estimated that there is one Ascarid present for every 1,000 eggs per gram of feces. It is also considered that there is an equal number of male and female worms.

Table IV shows the distribution of fertilized eggs according to the above intensity grouping. By computing the arithmetic mean in each intensity group the average percentage of fertilized eggs can be determined.

It can be seen from this analysis that the percentage of fertilized eggs increases with the intensity of infestation. It is also interesting to note that in the 289 cases in which unfertilized eggs occurred alone 265 of these or 91.7 per cent had light infestations, that is five female worms or less. This is the group in

TABLE I

ESTIMATED PER CENT OF FERTILIZED EGGS IN EACH SPECIMEN

GROUP	PER CENT FERTILIZED EGGS	GROUP	PER CENT FERTILIZED EGGS
1	0	5	60
2	15	6	75
3	35	7	90
4	50	8	100

TABLE II

PER CENT OF FERTILIZED AND UNFERTILIZED ASCARIS EGGS IN 1103 SPECIMENS OF FECES

TYPE OF EGG	NUMBER OF SPECIMEN	PER CENT
Unfertilized	289	26.2
Fertilized	355	32.2
Unfertilized and fertilized	459	41.6
Total	1103	100.0

which the diagnosis is most likely to be missed if unfertilized Ascaris eggs are not recognized. In those very light infestations in which only unfertilized eggs were present, there was an average of 2,300 eggs per gram of feces. In those specimens containing only fertilized eggs there was an average of 4,500 eggs per

TABLE III

CLASSIFICATION OF INTENSITY OF INFESTATION OF ASCARIS

INTENSITY OF INFESTATION. EGGS PER GRAM OF FECES	APPROXIMATE NUMBER OF FEMALE WORMS	CLINICAL CLASSIFICATION
0 - 9,999	0 - 5	Very light
10,000 - 49,999	6 - 25	Light
50,000 - 99,999	26 - 50	Moderate
100,000 - 299,999	51 - 150	Heavy
300,000 and over	151 and over	Very heavy

TABLE IV

INTENSITY OF INFESTATION, PERCENTAGE OF FERTILIZED EGGS IN EACH GROUP

INTENSITY OF INFESTATION. ESTIMATED NUMBER OF FEMALE ASCARIDS	PER CENT OF FERTILIZED EGGS								TOTAL NO. OF SPEC.	AVERAGE PER CENT FERTILIZED EGGS
	0% NO. OF SPEC.	15% NO. OF SPEC.	35% NO. OF SPEC.	50% NO. OF SPEC.	60% NO. OF SPEC.	75% NO. OF SPEC.	90% NO. OF SPEC.	100% NO. OF SPEC.		
0 - 5	265	5	25	24	20	24	13	178	554	41.8
6 - 25	24	15	20	21	29	55	92	138	394	76.0
26 - 50	0	8	4	6	6	14	42	25	105	78.5
51 - 150	0	1	1	1	2	5	20	14	44	86.2
151 and over	0	0	0	0	0	0	1	5	6	98.3
Total	289	29	50	52	57	99	172	355	1103	

gram of feces. When there are more than five female worms present, there is a progressive increase in the number of fertilized eggs.

DISCUSSION

The diagnosis of Ascaris lumbricoides by the examination of feces is not difficult when fertilized eggs are present in the specimen. The fact that not only fertilized but also unfertilized eggs may be found either alone or in the same specimen in ascariasis introduces a possible error in the diagnosis of this condition. If unfertilized Ascaris eggs are unrecognized, the degree of error may be large as in this series; 26.2 per cent of the specimens contained unfertilized eggs alone. In diagnostic laboratories where routine stool examinations are made, the diagnosis may be missed if these eggs are not recognized. In making extensive intestinal parasite studies, the information concerning the prevalence of Ascaris may not be accurate if this factor is not taken into consideration.

SUMMARY

1. A differential egg-count analysis was made on 1,103 specimens containing eggs of Ascaris lumbricoides.

2. In this series 26.2 per cent of the specimens contained unfertilized Ascaris eggs alone, 32.2 per cent contained fertilized eggs alone and 41.6 per cent contained both fertilized and unfertilized eggs.

3. The relative percentage of fertilized Ascaris eggs increases with the increase in the intensity of infestation.

4. The smallest number of fertilized eggs were found in the very light infestations, one to five female worms. When more than five female worms are present there is a progressive increase in the percentage of fertilized eggs.

A STUDY OF FRÖHDE'S TEST FOR MORPHINE*

CHARLES F. POE, PH.D., AND PEARL SURBRUGG STEHLEY, PH.C., M.S.,
BOULDER, COLO.

FRÖHDE'S test, which depends upon the reduction of a molybdate in acid solution, is one of the earliest and most used tests for morphine. With this alkaloid, Fröhde's reagent gives a reddish violet† color soon changing to blue, then to green, yellow, and light red. The test was first proposed by Fröhde in 1866,[1] and as a test with the alkaloids has been used frequently by various investigators.

Witthaus[2] lists a number of alkaloids and glucosides which give a red or violet color with this reagent, but states that all except toxoplerygin give the same color with the sulphuric acid alone. Rojahn and Struffmann[3] tried a number of alkaloids and glucosides with Fröhde's reagent. They found that arbutine and strophanthine give a violet coloration.

Miller and Taylor[4] and Levine and Yahr[5] tested a number of organic compounds by heating them with a dilute solution of a molybdate and sulphuric acid. A number of these gave color reactions. However, in the regular test for morphine with Fröhde's reagent no heat is used, and the reagent contains no water.

The object of the investigation reported in this paper was to test a large number of organic compounds with Fröhde's reagent in order to determine whether or not there were any great number of substances which would give the morphine test, and whether or not this test was characteristic of any given organic grouping, or class of organic compounds. Also, these organic compounds were mixed in varying amounts with morphine, and the test applied in order to determine which substances would interfere with the test.

PROCEDURE

The organic compounds, numbering over 450, and the methods used were approximately the same as those employed in a similar study of strychnine.[6]

Preparation of Fröhde's Reagent: One gram of finely ground sodium molybdate was dissolved in 100 c.c. of chemically pure concentrated sulphuric acid with the aid of heat.

Preparation of Standard Morphine Solution: A solution was prepared in alcohol containing one milligram of morphine per cubic centimeter.

Preparation of Solutions to be Tested: The compounds to be tested were dissolved in the proper solvent so that one cubic centimeter of the solution was equivalent to one milligram of the compound. Care was taken in selecting a suitable solvent which would not only readily dissolve the compound, but would also volatilize on the water-bath. Substances which were relatively insoluble were finely ground and suspended in some volatile solvent. The bottle was shaken before removing a portion of the contents to insure an even suspension of the substance to be tested.

Procedure of Tests: Three tests were made on each compound. For the first test, one cubic centimeter of the solution to be tested was evaporated to dryness and then tested with three or four drops of Fröhde's reagent, and any color change was recorded. In the second

*From the College of Pharmacy and Department of Chemistry, University of Colorado.
Received for publication, March 14, 1932.
†Most text books describe the color as purple or violet.

test, one cubic centimeter of the solution to be tested and one cubic centimeter of standard morphine solution were mixed and the test made as described above. If there was no interference, a reddish violet color resulted which soon changed to green. If there was an interference in the color reaction, the colors resulting were recorded. The third test was carried out as above, except that five cubic centimeters of the solution of the organic compound were used with one cubic centimeter of the morphine solution.

The results of the tests when the organic compounds were treated with the reagent are given in the pages immediately following. The name of the organic compound is given first, and directly opposite is given the color reaction. In cases where no color was developed, the compounds are listed with the statement, "No color reaction."

Alkaloids and Alkaloidal Salts

ORGANIC SUBSTANCES	COLOR REACTION
Aconitine	Blue to green
Apomorphine	Green to blue, trace violet
Belladonnine	Brown
Berberine	Dirty brown
Brucine	Reddish violet to blue
Codeine	Green to blue
Colchicine	Brownish orange
Cotarnine hydrochloride	Brick red
Cryptopine	Violet to green
Delphinine	Brown, purple streaks
Dionine	Greenish yellow
Emetine	Brown, trace violet
Heroine	Reddish violet
Hydrastine	Green to brown
Morphine	Reddish violet to green
Narceine	Brown to green
Papaverine	Light violet to green
Phenacaine	Light green
Piperine	Reddish orange to brown
Quinine	Pale green
Sanguinarine nitrate	Brown, trace purple
Solanine	Yellow
Veratrine	Yellow to orange

No Color Reaction.—Atropine, caffeine, cinchonidine, cinchonine, cocaine, daturine, ergotine, beta-eucaine hydrochloride gelseminine, homatropine, hyoscyamine, nicotine, pelletierine, physostigmine, pilocarpine, pseudopelletierine, quinidine, scopolamine, sparteine, strychnine, theobromine, theophylline.

Amino Acids and Derivatives

para-Aminophenylglycine	Dull green
Aspartic acid	Tan
Diiodotyrosine	Greenish grey
Edestine	Yellow
Glycyltryptophane	Greenish orange
para-Nitrophenylglycine	Brown
Phenylglycine	Brown
Tryptophane	Dirty green
Tyrosine	Blue

No Color Reaction.—Acetylphenylglycine, alpha-alanine, arginine, asparagine, dl-benzoylalanine, betaine hydrochloride, creatine, creatinine, ethylglycollate, glutamic acid, glycine, hippuric acid, isoleucine, leucine, alpha-phenylalanine, beta-phenylalanine, dl-valine.

Aliphatic Acids

Palmitic acid	Dirty brown

No Color Reaction.—Aconitic acid, adipic acid, dl-alpha-aminoacetylacetic acid, alpha-bromopropionic acid, beta-bromopropionic acid, formic acid, fumaric acid, levulinic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mucic acid, propionic acid, stearic acid, succinic acid, tartaric acid, tartronic acid, trichloroacetic acid.

Aliphatic Acid Salts, Esters, and Derivatives

No Color Reaction.—Ethyl oxalate, ethyl succinate, isoamyl propionate, isobutyl acetate, isobutyl isothiocyanate, methyl isothiocyanate, sodium formate, sodium oxalate, thallous formate, thallous malonate, triacetin, tributyrin.

Aliphatic Alcohols and Ketones

Methyl heptenone	Dirty yellow
Phorone	Tan

No Color Reaction.—Acetylacetone, cetyl alcohol, dulcitol, erythritol, ethylene glycol, isobutyl alcohol, isopropyl alcohol, mannitol, melissyl alcohol, octyl alcohol, trichlorobutyl alcohol.

Sugars

Levulose	Light yellow
Melezitose	Pale orange
Xylose	Grey

No Color Reaction.—Arabinose, galactose, glucose, lactose, maltose, d-mannose, raffinose, rhamnose, sucrose.

Urea and Uric Acid Derivatives

Allylphenylthiocarbamide	Yellow

No Color Reaction.—Allylthiocarbamide, allylthiourea, amytal (isoamylethylbarbituric acid), acetylmethylurea, allantoine, alloxantine, barbital (diethylmalonylurea), barbituric acid, biuret, dl-n-butylthiourea, dibromobarbituric acid, diphenylthiourea, guanine hydrochloride, ipral (calcium ethylisopropylbarbiturate), luminol (phenylethylbarbituric acid), peralga (aminopyrinediethylbarbiturate), thiobarbituric acid, thiourea, triphenylguanidine, urea, urethane, uric acid.

Glucosides

Arbutin	Violet
Colocynthin	Reddish brown to purple
Convallamarin	Brown
Digitalin	Magenta, streaks of purple
Elaterin	Olive green
Phloridzin	Navy blue
Picrotoxin	Yellowish orange
Salicin	Violet-blue to rose
Saponin	Greenish blue, streaks of violet

No Color Reaction.—Aesculin, amydalin, santonin.

Miscellaneous Aliphatic Compounds

Aminoguanidine bicarbonate	Dirty green
Bromoform	Greenish yellow
Isobutylbromide	Slight yellow
Oxamide	Grey

No Color Reaction.—Acetal, acetaldoxime, acetamide, acetoxime, aldehydeammonia, terbutyl bromide, chitin, chloral hydrate, chloral urethane, chloropicrine, dimethylglyoxime, hexachloroethane, hexamethylenetetramine, iodoform, methylglyoxal sodium bisulphite, monochlorohydrine, nitrosodiethylamine, oenathol, pinacol hydrate, propionamide, sulphonal, thialdine, tribromohydrine, trichloroacetylchloride, trimethylene bromide, trional, veronal.

Benzene and Toluene Derivatives

Azoxybenzene	Deep yellow
Mesitylene	Yellowish brown

No Color Reaction.—ortho-Bromochlorobenzene, para-bromochlorobenzene, ortho-bromonitrobenzene, meta-chloronitrobenzene, ortho-chloronitrobenzene, para-chloronitrobenzene, parachlorotoluene, ortho-dichlorobenzene, 2, 5-dichloronitrobenzene, iodosobenzene, isopropylbenzene, meta-nitrotoluene, ortho-nitrotoluene, para-nitrotoluene, styrene, para-xylenesulphonic acid.

Aniline and Derivatives

Acetyl-ortho-methyltoluidine	Greenish yellow
Aminoazobenzene	Dark brown
meta-Anisidine	Grey
ortho-Anisidine	Grey
para-Anisidine	Blue to grey
meta-Bromoaniline	Brown, traces of violet
ortho-Phenetidine	Grey, streaks dirty purple
para-Phenetidine	Grey, streaks dirty purple
ortho-Tolidine	Brown
meta-Toluidine	Greenish brown
para-Toluidine	Brown

No Color Reaction.—Acetoanilide, acetyl-para-anisidine, acetyl-para-methyltoluidine, acetylphenetidine, acetyl-ortho-toluidine, acetyl-para-toluidine, meta-acetxylidine, 2-aminotoluene-4-sulphonic acid, 2-aminotoluene-5-sulphonic acid, 4-aminotoluene-2-sulphonic acid, aniline, benzanilide, ortho-benztoluide, para-benztoluide, para-bromoacetanilide, ortho-bromoaniline, para-bromoaniline, chloramine, meta-chloroaniline, ortho-chloroaniline, para-chloroaniline, 1, 2, 4-dichloroaniline, 2, 5-dichloroaniline, 1, 2, 4-dinitroaniline, exalgine, meta-nitroaniline, para-nitroaniline, meta-nitrodimethylaniline, para-nitrodimethylaniline, nitrosodimethylaniline, 1, 2, 3-nitrotoluidine, 1, 2, 4-nitrotoluidine, 1, 3, 4-nitrotoluidine phenyl-beta-diphenylamine, ortho-toluidine, tribromoaniline, trinitroaniline, 1, 2, 4-xylidine, 1, 3, 4-xylidine.

Phenols and Derivatives

Acetyl-para-aminophenol	Pale blue
ortho-Aminophenol	Reddish violet
para-Aminophenol	Dirty green
5-Benzalamino-2-cresol	Bright green
para-Benzalaminophenol	Light green
Benzoylthymol	Greenish rose
Carvacrol	Brownish green, streaks violet
Catechol	Grey
ortho-Chloromercuriphenol	Bluish green
Phloroglucinol	Tan
Pyrogallic acid	Black, streaks violet

No Color Reaction.—Acetyl-meta-aminophenol, meta-aminophenol, para-bromophenol, ortho-chlorophenol, para-chlorophenol, meta-cresol, ortho-cresol, para-cresol, 3, 5-dibromo-orthocresol, 2, 4-dichlorophenol, dimethylhydroresorcinol, 2, 3-dinitrophenol, 2, 4-dinitrophenol, 2, 6-dinitrophenol, meta-nitrophenol, ortho-nitrophenol, para-nitrophenol, orcinol, phenol, picric acid, tetrabromo-ortho-phenol, thymol, tribromophenol, trichlorophenol, xylenol.

Aromatic Acids

Benzilic acid	Rose, trace of purple
Cinnamic acid	Yellowish green
Cumaric acid	Green to grey
Gallic acid	Green
Tannic acid	Brownish green

No Color Reaction.—Acetylsalicylic acid, meta-aminobenzoic acid, para-aminobenzoic acid, dl-aminophenylacetic acid, anisic acid, anthranilic acid, arsanilic acid, meta-bromobenzoic acid, ortho-bromobenzoic acid, para-bromobenzoic acid, meta-chlorobenzoic acid, ortho-chlorobenzoic acid, para-chlorobenzoic acid, cinchophen, diiodosalicylic acid, diphenylacetic acid, 5-iodosalicylic acid, mandelic acid, para-mercurichlorobenzoic acid, metanilic acid, naphthionic acid, meta-nitrobenzoic acid, ortho-nitrobenzoic acid, para-nitrobenzoic acid, quinic acid, salicylic acid, terephthalic acid, ortho-toluic acid, para-toluic acid.

Aromatic Acid Derivatives

Methyl cinnamic ester	Greenish blue
Neocinchophen (ethyl-6-methyl-2-phenylquinoline-4-carboxylate)	Yellowish green
Phenyl salicylate	Greenish grey

No Color Reaction.—Benzamide, benzoic anhydride, benzyl benzoate, butyl benzoate, cumarin, ethyl benzoate, ethyl salicylate, isoamyl benzoate, isoamyl salicylate, isobutyl benzoate, methyl benzoate, methyl salicylate, nicotinic acid nitrate, meta-nitrobenzoyl chloride, para-nitrobenzoyl chloride, phthalimide.

Aromatic Aldehydes, Ethers, Alcohols, and Ketones

Anisaldehyde	Dirty green
Benzhydrol	Reddish orange
1, 2, 5-Bromosalicylaldehyde	Greenish yellow
ortho-Nitrobenzaldehyde	Greenish yellow
Phthalic acid aldehyde	Tan
Salicylaldehyde methyl ether	Brown
Saligenin	Violet to blue
para-Tolylaldehyde	Black with violet
Vanillin	Greenish yellow

No Color Reaction.—para-Aminoacetophenone, anisole, benzalacetone, benzalacetophenone, benzophenone, ortho-bromoanisole, para-bromoanisole, ortho-bromonitrobenzaldehyde, ortho-chlorobenzaldehyde, isophthalicaldehyde, meta-methoxysalicylaldehyde, methylacetophenone, para-nitroanisole, 5-nitrosalicylaldehyde, phenetole, piperonal, salicylaldehyde, tetramethyldiaminobenzophenone.

Heterocyclic Compounds

Acridine	Yellowish green
Furoic acid	Pink
Isatin	Orange

No Color Reaction.—Antipyrine, dimethylpyrone, 6-nitroquinoline, oxyquinoline, piperidine, quinaldine, quinoline, skatole, succinimide.

Hydroaromatic Compounds

Carvene	Yellowish brown
Limonene	Brownish black
Terpenyl acetate	Tan

No Color Reaction.—d-Borneol, l-borneol, dl-camphor (natural), camphor (synthetic), camphoric acid, camphorsulphonic acid, carvenone, menthol, quercite, terpineol.

NAPHTHALENE AND ANTHRACENE DERIVATIVES

Acet-alpha-naphthalide	Greenish grey
Acet-beta-naphthalide	Greenish brown
Alizarin	Magenta, trace of purple
Dibromoanthracene	Light green
alpha-Naphthylamine	Bluish green
alpha-Naphthylaminoazobenzene	Black with violet
alpha-Naphthylisocyanate	Olive green

No Color Reaction.—Acenaphthene, alpha-bromonaphthalene, 1, 5-dinitronaphthalene, beta-naphthalenesulphonic acid, naphthalic anhydride, beta-naphthol, naphtholmethyl-alpha-ether, beta-naphthylamine, nitroso-beta-naphthol.

MISCELLANEOUS AROMATIC COMPOUNDS

Abietic acid	Brown
Adrenaline	Blue to green
Amarine	Bright green
Benzcatechin	Greenish brown, trace of purple
Benzoin	Pink
Benzylphenylhydrazine	Grey-blue to violet
Isoamylphenylhydrazine	Greenish grey
Isoeugenole	Brown-black
Phenanthrene	Deep orange
Phenanthrenequinone dioxime	Brown, streaks of purple
Phenolphthalein	Reddish orange
meta-Phenylenediamine hydrochloride	Greenish blue
Phenylhydrazine hydrochloride	Light green
Rheumatine (saloquinine salicylate)	Yellowish green
Salvarsan	Green
Tetrabromophenolphthalein	Rose
Thymolphthalein	Magenta
Triphenylmethane	Bluish green
Turmeric	Brownish purple

No Color Reaction.—Benzil, 2, 4-dinitrophenylhydrazine, diphenyl, fluorene, ortho-nitro-acet-meta-xylidide, meta-nitrobenzhydrazide, para-nitrophenylhydrazine, quersite, thiosemicarbazide, ortho-tolunitrile, para-tolunitrile, para-tolylisothiocyanate, para-tolylquinoline-sulphate, para-tolylthioquinanthrene.

The color reactions for the tests where an equal amount of impurity was added to the morphine were recorded, and also the reactions where five times the amount of impurity was added; but the listing of these would require too much space. Therefore, only those reactions in which the reddish-violet color was completely masked will be given. The list of substances previously given may be referred to in order to obtain the names of the compounds which did not completely cover up the morphine test. Many of these gave no interference, but others gave more or less interference.

Organic compounds which completely covered up the morphine test when present in equal amounts were: Azoxybenzene, para-aminoazobenzene, adrenaline, benzalacetophenone, and nicotinic acid nitrate.

Organic compounds which completely covered up the morphine test when present in amounts five times that of morphine were: Azoxybenzene, para-amino-azobenzene, para-aminophenol, adrenaline, benzalacetophenone, benzhydrol, beta-

naphthylamine hydrochloride, nitrosodiethylamine, nicotinic acid nitrate, piperine, phenolphthalein, and sanguanine nitrate.

A study of the preceding data shows that there were twenty-nine compounds which gave various shades of violet, lavender, or purple, which might, more or less, be confused with the Fröhde's test for morphine. However, over a fourth of the compounds gave some color test with the reagent. In general, the compounds giving a similar color, as does morphine, with this reagent do not belong to any definite group of organic compounds, nor does any special organic radical seem to be responsible for the characteristic test. As a group, the alkaloids seem to give more of the positive test than does any other group.

There were very few organic compounds which completely masked the morphine test. However, there were many interferences to a greater or less degree. Of course, in a carefully conducted examination for morphine many of the foreign organic compounds would be removed by means of different organic solvents.

CONCLUSIONS

1. A number of organic compounds have been found to give a color test similar to that given by morphine with Fröhde's reagent.

2. The interferences of organic compounds with Fröhde's test for morphine have been determined when present in varying amounts.

REFERENCES

1. Fröhde: Ann. d. Chem. **120**: 188, 1861.
2. Witthaus, R. A.: Manual of Toxicology, ed. 2, 1911, p. 998.
3. Rojahn, C. A., and Struffmann, F.: Examination of Pharmaceutical Specialties and Nostrums, Pharm. Zentralbl. **70**: 325, 1929.
4. Miller, C. W., and Taylor, A. E.: Reduction of Ammonium Molybdate in Acid Solution, J. Biol. Chem. **17**: 531, 1914.
5. Levine, V. E., and Yahr, H. M.: Reduction With Compounds of the Rarer Elements, Biochem. Bull. **4**: 224, 1915.
6. Poe, C. F., and O'Day, D. W.: A Study of Mandelin's Test for Strychnine, J. Am. Pharm. Assn. **19**: 1292, 1930.

THE LOCAL IMMUNIZATION OF THE PERITONEUM BY THE USE OF BACTERIOPHAGE. A NEGATIVE REPORT*

THURMAN B. RICE, M.D., INDIANAPOLIS, IND.

IN THE course of a long series (approximately 500) of suppurative cases treated by local application of bacteriophage under the direction of the author[1] it was found that appendiceal and other abscesses in the abdominal cavity, due to Staphylococcus aureus and Escherichia coli did very well after irrigation with bacteriophage filtrates. Some of our best results were those with persistent abscess cavities discharging large amounts of pus. In nearly every such case the cavity rapidly cleaned itself and began to heal rapidly. In a few cases which were particularly suitable for such study and in which the patient had a low blood leucocyte count there was a marked increase in the number of white cells in the circulating blood,

*From the Department of Bacteriology, Indiana University School of Medicine.
Received for publication, March 22, 1932.
[1]Rice: Am. J. M. Sc. **179**: 345, 1930.

after the local administration of bacteriophage by instillation into the abscess cavity.

Inasmuch as it is better to prevent infection than attempt to treat it, the thought came to us that it might be possible to immunize the peritoneum by injecting into the peritoneal cavity a mixture of staphylococcus—streptococcus—B. coli bacteriophage filtrates. Such a procedure if successful would apparently be of much value as a prophylactic measure to precede an abdominal operation likely to result in contamination of the peritoneal cavity. Experiments upon man being manifestly out of the question, the dog was chosen as a suitable experimental animal.

It was easily possible to make preliminary experiments upon animals that were being used by the Senior class of Indiana University School of Medicine in the course in Operative Surgery on the dog under the direction of Dr. W. D. Little. These dogs were aseptically operated upon under ether anesthetic. After the required experiments were done the students were asked to tie off the cecum with sterile silk sutures before closing the abdomen. Half of the dogs had been given an injection of Staph-strep-coli "phage" on the three days immediately preceding the operation while the others had had no experience with it. It seemed that the animals with "phage" did better than those without, but the operations in the hands of inexperienced students were not sufficiently accurate to enable us to make sure of our conclusions.

Five series of much more accurate experiments were planned to follow the preliminary tests just described. Thirty dogs were operated upon under the most careful aseptic technic that could be arranged. Half of these animals had been given bacteriophage intraperitoneally at some time preceding or during the operation, while the other half had had none. Dogs were chosen in pairs which were as much alike as possible in age, size, breed, and general condition. One of each pair was used as a test animal and the other as a control which was handled in every way like the test animal except with regard to the giving of the bacteriophage.

The skins of the dogs were washed thoroughly with soap and clean warm water, and then shaved or deprived of hair with a depilatory. The clean skin was then thoroughly scrubbed, dried with alcohol followed by ether and then painted with tincture of iodine which was later washed off with alcohol. A clean and freshly sterilized set of instruments was used on each animal. Sterile drapings were used in abundance and every possible precaution taken to prevent contamination. After closure the dogs were placed in a pen by themselves and watched closely. The first animals had their temperatures and white cell counts taken at frequent intervals but this was discontinued after the first twelve animals, as it did not seem to give information sufficiently valuable to compensate for the great amount of work in making these determinations. It seems that dogs vary widely in these respects and that a great mass of information would be needed to interpret such findings. All dogs were subjected to autopsy as soon as possible after death and those that lived were killed during or rather soon after convalescence so that the condition of the abdomen might be ascertained.

Original protocols are given:

Series I.—Twelve dogs, six tests and six controls. The test dogs in this experiment had been given 30 c.c. of mixed Staph-strep-coli phage on each of the three days preceding the operation and

then had the exposed cecum wrapped in a phage-saturated gauze sponge for fifteen minutes at the time of operation, and had 15 c.c. of the phage poured over the cecum and into the abdominal cavity. At the time of operation one and one-half inches of the empty cecum were tied off with sterile silk thread.

Dog II.—(Test) Killed after five days while apparently very ill. Marked generalized peritonitis with much bloody exudate. Few adhesions, and little plastic exudate. Cecum completely gangrenous. No evidence of local abscess formation.

Dog I.—(Control) Killed after five days while apparently in good condition, certainly better condition at least than the corresponding test animal. There was a large abscess in the abdominal wall just beneath the cecum (pus was brown in color and slimy). Purulent (not bloody) exudate in the abdominal cavity. Generalized peritonitis. Cecum gangrenous and with a definite abscess wall about it. Apparently the abscess had been ruptured (dog had jumped from a high table the day before). *Control in better condition than test animal.*

Dog III.—(Test) Died after forty hours. Bloody exudate in peritoneum, some fibrinous exudate. No adhesions of consequence. Cecum not at all walled off, gangrenous and nearly digested.

Dog IV.—(Control) Killed after five days while in fairly good condition. There was some evidence of peritonitis. The omentum was tightly adherent to everything in the region of the cecum. Upon tearing away these adhesions the cecum was found to be about the size of a hen's egg, considerably darker than the surrounding viscera, tight and distended, but apparently *not* gangrenous. There was no evidence of general *peritonitis.* Apparently nature had succeeded in preventing the spread of infection. *Control in much better condition than the test animal.*

Dog VI.—(Test) Killed after fourteen days being at that time apparently in good condition. The omentum was found firmly adherent in many places. Loops of the bowel were also firmly joined. No evidence of active peritonitis at time of autopsy. In one place a well walled off abscess with thick creamy pus was found (1 c.c. of pus). This abscess was at the former site of the cecum as was proved by the fact that the black silk suture was found in the abscess cavity.

Dog V.—(Control) Killed after fourteen days while apparently in good condition. Upon opening the abdomen there was no evidence of general peritoneal irritation. The omentum was adherent in only one or two places. The bowel loops were not adherent except in the region of the cecum. About the region of the cecum was a very tight knot of adhesions, but it was possible to make out the stump of that organ. The tied off portion was completely destroyed. There was no evidence of pus or recent active inflammation. *The control was in somewhat better condition than the test animal.*

Dog VII.—(Test) Died after forty hours. The abdomen contained a large amount of bloody exudate. Cecum was gangrenous and ruptured. There was a small amount of plastic exudate on the bowels, but no effective walling off. Omentum badly congested, somewhat sticky but not tied up in adhesions.

Dog VIII.—(Control) Killed at forty-four hours. The peritoneal cavity contained no fluid exudate. Omentum rather firmly adherent about the cecum about which it forms a ball. Cecum was found tightly tied off but only slightly redder than the surrounding viscera. It was definitely not gangrenous. There was no abscess cavity about it and the remaining peritoneum was essentially normal in appearance. It was evident that the blood vessels from the omentum had succeeded in nourishing the tied off cecum. *Control very much better than the test animal.*

Dog X.—(Test) Died after forty-four hours. Abdomen contained a large amount of bloody exudate. Bowels greatly congested, and everywhere covered with a fibrinous exudate. The omentum covered the bowels and was rather well arranged about the cecum. There was a definite abscess cavity (beginning) about the gangrenous cecum.

Dog IX.—(Control) Killed after forty-four hours while apparently in fair condition. The peritoneum seemed comparatively normal. There was no exudate and no adhesions except about the cecum. In this region there was a bunch of massed adhesions showing little inflammation on the outside. By breaking down the friable adhesions we find exactly in the center of the mass the gangrenous but not ruptured cecum. *The control animal was in much better condition than the test animal.*

Dog XI.—(Test) Killed after five days while apparently in normal condition. There was no evidence of peritonitis. Loops of bowel not at all adherent except in the region of the cecum

where there was a mass the size of a lemon. The center of this mass contained the cecum which was *not* gangrenous and only a little darker than the surrounding normal bowel. Blood vessels of considerable size could be seen passing from the omentum to the tied off cecum. Apparently this animal would have lived.

Dog XII.—(Control) Died after five days. Marked peritonitis with hemorrhagic exudate and fluid in the peritoneal cavity. Cecum gangrenous and practically digested. No evidence of walling off. *In this case the control was in much worse condition than the test animal.*

Résumé of results in Series I.—In five instances the test animal fared worse than the control. It seemed that the bacteriophage tended to prevent the plastic adhesions which would have sealed off the danger area. It seemed also to digest the devitalized tissue as is shown by the fact that the cecum in most of the test animals was markedly more disintegrated than in the controls. Bacteriophage was not able to control the peritonitis produced.

Criticism of this series might be made on the grounds that the bacteriophage was given in too large dosage and that it irritated the peritoneum previous to the operation. This was not the case so far as could be seen at the time of the operation, the two sets of animals being indistinguishable on this score. Various experimenters have shown that bacteriophage tends to attract leucocytes and to cause the rapid solution of suppurative tissue. This is probably the reason why infected areas liquefy and clean themselves so rapidly. It seems likely that this action prevented the formation of the protective adhesions which would have prevented spreading of the peritonitis.

Series II. Six dogs—Three tests and three controls. The test dogs had 30 c.c. of mixed Staph-strep-coli bacteriophage at the time of operation. The cecum was exposed, 3 to 5 c.c. of fecal material was pressed into it from the bowel. It was then tied off and then punctured. The fecal matter was pressed out into the peritoneal cavity in the region of the cecum but was not spread about. The hole in the cecum was then closed with purse string suture and turned in.

Dog XIV.—(Test) Died after twenty-four hours. There was a small amount of fluid exudate, moderate generalized peritonitis, no plastic exudate. Cecum was well closed, gangrenous, no adhesions about it.

Dog XIII.—(Control) Killed after eight days while apparently in good condition. The omentum was slightly adherent to bowels and the bowels slightly adherent to each other in places except in region of the cecum where there was a ball about that organ. No evidence of pus or active peritonitis. *Control better than test.*

Dog XVI.—(Test) Died after twenty-four to thirty-six hours. Moderate generalized peritonitis. Very small amount of fluid exudate; no plastic exudate. Cecum well closed, no adhesions about it.

Dog XV.—(Control) Died after twenty-four hours. Marked peritonitis, small amount of fluid exudate, moderate amount of plastic exudate. Cecum closed, no adhesions about it. *Control and test essentially the same.*

Dog XVIII.—(Test) Died after twenty-four hours. Marked generalized peritonitis. No fluid exudate, no plastic exudate, no adhesions. Cecum closed.

Dog XVII.—(Control) Killed after eight days while apparently in good condition. There were signs of generalized peritonitis still somewhat active. There was an enormous mass of adhesions about the cecum. There was a pus cavity (2 to 3 ounces) in the region of the cecum but it was well walled off. *Control animal did considerably better than the test.*

Résumé of Series II.—Again the control animals recovered considerably better than those used as tests. Conclusions reached after Series I are strengthened. The bacteriophage seems to prevent walling off of the involved area but is unable to control the peritonitis.

Series III. Six dogs—Three test and three controls. The test animals were given mixed Staph-strep-coli bacteriophage (30 c.c.) three, two, and one days before operation, but none at the time of operation. The cecum was squeezed full of fecal material from the bowel and then tied one and one-half inches from the end. It was not punctured.

Dog XIX.—(Test) Died after seventy-two hours. Marked generalized peritonitis, considerable quantity of fluid in the abdomen. Cecum gangrenous but not ruptured until manipulated

at autopsy. This animal had been closed at operation with difficulty and there was a large infected area in the line of closure. This abscess contained a large amount of fluid pus and seemed in itself to be sufficient to cause death.

Dog XX.—(Control) Died after forty hours. Very violent peritonitis. Cecum gangrenous. Much bloody exudate. A few adhesions between omentum and bowel. *Test animal fared somewhat better than the control.*

Dog XXI.—(Test) Died after seven days. This animal seemed to be in good condition for several days after the operation and then changed for the worse and died. At autopsy the wound was broken down. Evidence of marked generalized peritonitis. Cecum gangrenous and ruptured. Small amount of plastic exudate at different places in the abdomen but little or no definite walling off.

Dog XXII.—(Control) Died after forty hours. Very marked generalized peritonitis. Large quantity of bloody exudate in the peritoneal cavity. Omentum and bowel somewhat adherent but no definite walling off. *Control worse than test.*

Dog XXIII.—(Test) Died after ninety hours. Marked generalized peritonitis. Copious bloody exudate, purulent. Few adhesions were about the cecum which was gangrenous but there was no real walling off.

Dog XXIV.—(Control) Died after seventy hours. Marked generalized peritonitis. No adhesions of any consequence. Considerable quantity of bloody purulent exudate. Cecum was gangrenous. *Control about like test.*

Résumé of Series III.—In this series there was little difference between test and control though in each case the test animal lived longer. Only in the case of Dog XXI was there, however, a significant difference. Evidently the large mass of fecal material in the cecum made recovery nearly impossible.

Series IV. Six dogs—Three test and three controls. In this series the test dogs were given 30 c.c. of mixed Staph-strep-coli bacteriophage on the third, second, and first day before operation, but none at operation. The empty cecum (emptied by squeezing into the bowel) was tied off.

Dog XXV.—(Test) This dog was in good condition after thirteen days and was used by mistake for another operative purpose. No autopsy was done but the animal was apparently doing well.

Dog XXVI.—(Control) Killed after twenty-seven days while apparently in good condition. Low grade peritonitis. About 700 c.c. of thin bloody fluid found. Mass of firm adhesions about cecum which had sloughed. Omentum injected. *Both did well.*

Dog XXVII.—(Test) Died after three days. Considerable quantity of bloody fluid in peritoneal cavity. Marked generalized peritonitis. Cecum gangrenous and ruptured, not walled off, no adhesions.

Dog XXVIII.—(Control) Died after four days. Marked generalized peritonitis with bloody purulent exudate. Cecum was gangrenous and ruptured. Some slight plastic exudate, no walling off. *No real difference between test and control animals.*

Dog XXX.—(Test) Died after three days. Thick bloody purulent exudate in peritoneal cavity. No plastic exudate except just about the cecum where the omentum was bunched up about the gangrenous cecum. Cecum was ruptured in pulling away the adherent bowel.

Dog XXIX.—(Control) Killed after twenty-seven days while in good condition. Many dense adhesions about the cecum. No fluid or evidence of active peritonitis. *Control did much better than the test animal.*

Résumé of Series IV.—Only in the last two animals was there a difference. In this case the control did much better than the test animal.

Recently Dr. Little's class has again had the opportunity to try out the same experiments in the Dog Surgery Class. The results this year confirm those reported here and repudiate those obtained by the class last year.

In the light of these experiments it seems that *bacteriophage is distinctly contraindicated in the abdomen when the diseased area is not walled off.* After a wall of plastic exudate has been formed and a definite pyogenic membrane has developed, we still believe that bacteriophage is excellent treatment. In that case, of

course, the filtrate does not at all enter the peritoneal cavity and apparently acts in exactly the same way in an abdominal abscess cavity that it acts in other abscess cavities.

It has been repeatedly noted that bacteriophage injected into an abscess causes a rapid liquefaction of the exudate thereby facilitating prompt and complete evacuation of the cavity. This action is so marked that occasionally physicians have objected to its use on the grounds that it caused too great a slough. We have felt however, that this criticism has not been well founded because the cavities have healed promptly as a rule and with a minimum of scar production. The mechanism of this liquefaction is suggested when we remember that bacteriophage stimulates the leucocytes as is shown by the increased flow of pus and increased white cell count in the blood.

This negative report is made in the hope that it will deter physicians from using bacteriophage in the abdomen either before or during the operation. We believe that *it should be used only after the diseased area is well walled off.*

SUMMARY

1. Staphylococcus-streptococcus-B. coli bacteriophage will not immunize the peritoneum as used in this experiment.

2. It will not prevent a peritonitis in dogs as a result of tying off the cecum when used at the time of operation.

3. It seems to inhibit the formation of walling off plastic exudates when used before or during the operation.

4. Experience other than that mentioned above indicates that it is of value in the treatment of abscesses already walled off whether they are in the abdominal cavity or elsewhere.

LOCALIZED AREAS OF ISCHEMIA ON THE HANDS*

WALLACE MARSHALL, B.A., AND CLEVELAND WHITE, M.D., CHICAGO, ILL.

HISTORICAL DATA

BIER,[1] in 1898, described multiple white spots which occurred on the hand after he had occluded the blood supply in the cubital region by means of a rubber constrictor. Since then, many workers have enlarged upon Bier's original findings, and have also described red spots as being present also. Gilding[2] has produced Bier's spots in animals. Wolf,[3] Boas,[4] Krogh,[5] Lewis,[6] and others have contributed much to our knowledge in respect to the intimate anatomy and physiology which is concerned in the production of these spots.

In 1919, Tracy[7] described chronic nonfactitious vasoconstriction spots in man and linked the presence of these spots to organic neural disease. Pigment spots were present at the periphery of the white spotted regions. Also the presence of anemic dermatography was noted as coexisting in some patients with white spots.

*From the Department of Dermatology, Northwestern University Medical School.
Received for publication, March 5, 1932.

ANATOMY AND PHYSIOLOGY

Spalteholz[8] claims that the number, and often the size, of the blood vessels to the skin, especially the palms of the hand and the soles of the feet, are greater in number than in other cutaneous areas. There exists an intimate anastomosis between the arteries which produce a cutaneous arterial network (Lewis). The subpapillary arterial network arises from the superficial network and proceeds outwards. Numerous small branches arise from the subpapillary network and thus form the terminal arterioles which supply the superficial layers of the skin and run parallel with it. Spalteholz claims that the cutaneous arterial network has thick muscular coats.

The vasomotor nerves, a part of the autonomic nervous system, enter the muscular coat of the blood vessels by means of their nonmedullated fibers. These vasomotor nerves constrict and dilate these cutaneous vessels. The vasomotor nerves either act by stimulation or depression of the vasomotor centers in the medulla and spinal cord, or they act by means of the humoral mechanism through the blood supply.

ETIOLOGY

It has been well established that vasoconstriction is responsible for these transient multiform white areas. It is our belief that this phenomenon may be but a further example of the spasmodic action of the blood vessels which is observed in other clinical diseases, such as Buerger's and Raynaud's diseases, erythromelalgia and angina pectoris, as well as in angiospasms of the brain, as has been described by Ten Broeck,[9] Osler,[10] and others. The action of the muscular contractions in asthmatics may also point to the above diseases as being of an anaphylactic nature. Angiospasms have anoxemia as their end result. These vascular spasms may be caused by the neurotropic action of the media in the blood vessels. Some observers claim that the hydrogen ion concentration is the basic cause. Other workers claim that this angiospastic action is the result of sclerotic vascular changes, or hypertension, or even both.

It is our belief that one of the main etiologic factors of the condition of localized ischemia in the hand, is the natural condition of the patient. These people presenting this lesion are of a nervous, high-strung type. Contrary to Tracy's belief, there was found no evidence of coexisting brain disease or other neural pathology. There was no vascular spasm in the feet. This may be due to the more marked muscular activity of the hands, more exposure to the elements, and the thinner cutaneous coverings of the hands.

CASE REPORTS

CASE 1.—A white male student, aged twenty-eight, exhibited some spots (white) which he had noticed on both hands. He had these patches as long as he could remember. Physical examination revealed no apparent pathology in any part of his body, except that he exhibited a chronic scaling of his scalp, a pronounced sinus arrhythmia (no correlation with the respiratory phases), and a persistent insomnia. His blood Wassermann was negative. His past and present health was good. None of his immediate family had the white spots on their bodies. Dermatologically, transient white macules were observed on both hands. These spots extended from the region of the wrists to the finger tips. These patches were present on the dorsal and ventral surfaces and were irregular in outline; the average was 4 to 8 mm. in diameter. It was observed that these multiple white spots had an average duration of approximately fifteen to forty-five seconds and

then disappeared only to reappear elsewhere in the region which we have described. These white macules would disappear when the surface of the hands were either gently rubbed or elevated above the head, and would rapidly reappear when the hands were hung at the patient's side.

CASE 2.—A white male, aged twenty-eight, whose previous occupation was that of an office worker, presented transient white macules which were continually present as long as he could remember. Physical examination revealed the patient to have a characteristic Parkinsonian syndrome of eleven years' duration. These spots had been noted previous to the onset of encephalitis. His blood Wassermann was negative. He experienced difficulty in walking, which was a part of his Parkinsonian. He had a decided scaling of his scalp, sinus arrhythmia (no correlation with the respiratory phases) and was troubled with insomnia. He states that his father also has the white spots on his hands. These multiple white macules were in the same location as in the other case. They average about 6 to 10 mm. in diameter. Their average duration was approximately one minute. They disappeared only to reappear in another location. Rubbing the hands or elevating them above the patient's head caused them to disappear, while lowering the hands to the patient's side caused them to reappear quite rapidly.

SUMMARY

1. Two cases of multiform, small areas of transient ischemia, which are continually residual in the patients, are described.

2. These white macules resemble Bier's spots and the macules described by Tracy as "chronic vasoconstrictor spots."

3. There was no evidence of the presence of associated pigment spots, nor was there any associated anemic dermatographý present in these patients.

4. It is believed that these spots occur in patients of a neurasthenic constitution. These patients showed a hyperactive nervous system manifested by sinus arrhythmia and insomnia. It is not our belief that these macules are associated with neural pathology, as Tracy has stated.

5. These multiform areas of localized ischemia on the hands are not uncommon, although not described in any of the standard dermatologic textbooks. They may be easily confused with vitiligo.

REFERENCES

1. Bier, A.: Der Ruckfluss des Blutes aus Ischamischen Korpertheilen, Arch. f. Path. Anat. **153**: 306-334, 1898.
2. Gildine, H. P., and Rous, P. J.: Studies of Tissue Maintenance, J. Exper. Med. **50**: 471-487, 1929.
3. Wolf, E. P.: Local Changes of Color in Skin Deprived of Its Normal Blood Supply, Heart **11**: 327-335, 1924.
4. Boas, E. P.: Capillaries of Extremities in Acrocyanosis, Blood Pressure and Morphology, J. A. M. A. **79**: 1404-1406, 1922.
5. Krogh, A.: The Anatomy and Physiology of Capillaries, New Haven, 1922, Yale University Press.
6. Lewis, T.: Blood Vessels of the Human Skin and Their Responses, London, 1927, Shaw and Sons.
7. Tracy, E. A.: Chronic-Vasoconstriction Spots and Their Significance, Boston M. & S. J. **180**: 1, 1919.
8. Spalteholz: (Quoted by Lewis. See above.)
9. Ten Broeck, L. L.: Vascular Disturbances, M. J. & Record (Supp.) **121**: 234-236, 1925; Cont. **121**: 274-277, 1925.
10. Osler, Wm.: Transient Attacks of Aphasia and Paralysis in States of High Blood Pressure and Arteriosclerosis, Canadian M. J. **1**: 919-926, 1911.

THE NONSPECIFIC FIXABILITY OF GUINEA PIG SERUM WITH BORDET'S ANTIGEN*

HESTER A. AUSTIN, B.S., ROCHESTER, N. Y.

THE possibility that the guinea pig serum used as complement in the Wassermann test might enter into nonspecific combination with the lipoidal antigens has been taken into consideration by all careful users of this reaction. Special mixtures to supply information on this point are often included as controls in the tests and titrations of complement. The usual controls, however, have not always been adequate, as they have been devised to test for nonspecific fixability of the complement chiefly with the cholesterinized antigen. The record of a recent experience, to be described in this paper, indicates that it is equally important to test for nonspecific fixability of complement with the acetone-insoluble fraction of heart muscle lipoids, known as the Bordet antigen.

The Rochester Health Bureau Laboratories follow very closely the New York State Laboratory Wassermann technic.[1] As a part of that technic, two controls on the anticomplementary properties of each antigen employed are used, 0.1 c.c., the same amount that is used in the routine fixation test and four times that amount, or 0.4 c.c. These controls are supposed to hemolyze after 15 minutes' incubation in the water-bath at 37° C., to show that the antigen is not anticomplementary. It was occasionally observed that while both controls for the cholesterinized antigen, and usually the 0.4 c.c. control of the Bordet antigen, cleared promptly, the 0.1 c.c. amount of Bordet antigen would fail to hemolyze.

It was also observed that occasionally most spinal fluids tested on a certain day, would give reactions with the Bordet antigen, while either none, or only those with a possible luetic history, would give reactions with the cholesterinized antigen. Serums tested on the same day failed to show this unusual result. It was found that these reactions of spinal fluid with the Bordet antigen took place on the days when the controls of the Bordet antigen were apparently anticomplementary.

The Bordet antigen used in these tests was an acetone-insoluble fraction of lipoids of beef heart muscle prepared at the New York State Health Department Laboratories. It is described in detail in "Standard Methods of the Division of Laboratories and Research of the New York State Department of Health" by Dr. Augustus B. Wadsworth in 1927[1] (pp. 182, 189), as follows:

"Remove the fat, blood vessels, pericardium, and endocardium, and cut up the muscle into cubes about one inch in diameter. Then grind the heart muscle in a meat chopper. Weigh the ground tissue, and for each 100 grams add 125 c.c. of 95 per cent alcohol.

"The purpose of the alcohol is to coagulate the proteins and not to dissolve

*From the Rochester Health Bureau Laboratories, Department of Bacteriology, School of Medicine and Dentistry, University of Rochester.
Received for publication, February 24, 1932.

the fatty substances or lipoids, and, as the alcohol is in comparatively small proportion and is immediately diluted with the tissue juice, it exerts but slight dissolving power.

"Let the mixture stand at room temperature for several days, then filter, spread the residue on trays in a thin layer and dry in the incubator for a day. To the dried tissue add 200 c.c. of acetone for each 100 grams of tissue originally used. Mix and leave at room temperature for one week, shaking for a minute or two once a day, then filter. Add 200 c.c. of fresh acetone for each 100 grams of tissue originally used, mix, and filter the next day. Remove the tissue to trays, and dry in the incubator for one day, then pulverize it finely in a mortar. To the ground tissue add 200 c.c. of 95 per cent alcohol for each 100 grams of tissue originally used. Mix and allow to extract at room temperature for ten days, shaking for a minute or two three times a day. Then remove the alcoholic extract by filtration through soft filter paper, and store in the dark at room temperature."

"Dilute the acetone-insoluble antigen as follows: Evaporate to dryness one part of antigen, by pipetting the antigen on a watch glass of about six-inch diameter or in an evaporating dish and placing it in front of an electric fan. Then suspend the dried residuum in two and one-half parts of distilled water. For example, if 3 c.c. of antigen is evaporated to dryness, suspend the dried residuum in 7.5 c.c. of distilled water. Add the first cubic centimeter slowly with a 0.2 c.c. pipette and mix as thoroughly as possible with a glass rod after the addition of each 0.2 c.c. The remainder of the water may be added rapidly, but make sure that the suspension is thoroughly mixed. Add the appropriate amounts of this suspension to the amounts of 0.85 per cent salt solution necessary to make the dilutions desired. Mix well by shaking."

In this laboratory the antigen is dried in an evaporating dish by means of a small stream of filtered compressed air.

The cholesterinized antigen, which rarely gave fixation tests with guinea pig serums, was an alcohol soluble fraction of lipoids from ether-extracted heart muscle, reinforced with 0.4 per cent cholesterin. It, also, was prepared at the New York State Health Department Laboratories in Albany. The procedure used in the preparation of this antigen is also described in "Standard Methods of the Division of Laboratories and Research of the New York State Department of Health" by Dr. Augustus B. Wadsworth in 1927[1] (pp. 182, 189), as follows:

"Cut and grind the heart muscle, as in preparing tissue for the acetone-insoluble antigen. Then spread it in the thinnest possible layer on a clean, smooth surface, such as a glass plate or stone shelf. Dry before an electric fan for five or six hours, then turn the tissue with a spatula and allow the other side to dry before the fan overnight. Break up the resulting dry sheet of tissue and grind it again. Spread this powder on a tray and dry it in an incubator at 37° C. for three or four days, then pulverize it finely in a mortar.

"Extract the dry, pulverized tissue with successive additions of ether, in the cold room, until the ether is practically water clear, filtering off the ether and replacing it with fresh ether daily. After the final ether extraction, allow the tissue to dry on a tray until the odor of ether is not evident.

"To prepare the alcoholic extract of the dried, ether-extracted tissue, add

10 c.c. of absolute alcohol per gram of tissue and boil for one hour under a reflux condenser. After cooling to room temperature, filter the extract through soft filter paper. In case a precipitate forms after standing, refilter before adding cholesterin.

"Cholesterinize 100 or 200 c.c. portions of this extract as needed, by adding 0.4 gram per 100 c.c. Weigh the cholesterin accurately on an analytical balance, place in a clean bottle, and add the measured quantity of alcoholic extract. Shake the mixture, and, if necessary, crush large particles of the cholesterin with a glass rod. Stopper the bottle tightly and place in the water-bath at 37° C. until the cholesterin is completely dissolved. Leave at room temperature for twenty-four hours and filter, if a precipitate is then evident.

"Dilute the cholesterinized antigen as follows: Pipette the antigen to the bottom of a one liter beaker. Place the required amount of 0.85 per cent salt solution in another liter beaker. Pour the salt solution onto the antigen as rapidly as possible and mix thoroughly by pouring back and forth from one beaker to the other, several times." (In this laboratory 100 c.c. bottles instead of one liter flasks are used.)

"Antigen, before use in complement fixation tests, is titrated for lytic, anticomplementary, and antigenic properties, the anticomplementary and antigenic properties being determined from titrations with at least twenty different specimens of pooled complement, during a period of at least four weeks. The dilution of optimum sensitivity, within the range which is antigenic but not lytic or anticomplementary, is then determined by titrations and tests of syphilitic sera."

Antigenic dilutions were never used under fifteen minutes, or over two hours, old in this laboratory.

The question arose as to whether this inhibition of hemolysis was due to an anticomplementary action of the antigen itself, or to the complement. In order to determine whether the inhibitory effect was due to the antigen, the following procedures were carried out: (1) Varying the length of time of drying the antigen before dilution, (2) Drying the antigen by compressed air, or by means of the electric fan, (3) Adding the total amount of distilled water in diluting, in 0.2 c.c. amounts, or by following the directions on the bottle, and adding only the first 1 c.c. in 0.2 c.c. amounts, (4) Prolonging emulsification in the distilled water, or fairly rapid emulsification, still following the directions on the bottle, (5) Using the stock normal salt solution, or using a special normal salt solution made by weighing the salt on the analytical balance, (6) Considering the possibility that the antigen dilution might be hemolytic for sheep cells, and that in the 0.4 c.c. antigen control, this hemolytic property might veil the anticomplementary property. To test this, 1-10, 1-20, 1-30, and 1-40 as well as the routine 1-50 dilution were made up; and 0.1 c.c. and 0.4 c.c. of each dilution were tested with 5 per cent cells. No hemolysis occurred even in the 1-10 dilution.

Varying these different conditions in the preparation of the antigen made no difference in the final result. This led to the conclusion that the factors producing the result must be in the complement.

It was decided, therefore, to test the individual guinea pig serums for nonspecific fixability with Bordet's antigen, in addition to the test which had always

been carried out routinely with the cholesterinized antigen, in conformance with the New York State Laboratory Wassermann technic. The test was performed according to the following directions: Prepare a 1-10 dilution of each guinea pig serum; use 0.1 c.c. serum dilution; add 0.2 c.c. antigen dilution; fix for two hours in the refrigerator at from 3° to 6° C.; remove from the refrigerator and add 0.2 c.c. sensitized cells; incubate in the water-bath at 37° C. until hemolysis is complete; record the time required for complete hemolysis with each guinea pig serum. Include in the pooled complement only the serums with which the sensitized cells are hemolyzed in less than ten minutes; the others show nonspecific fixability and are discarded as unsuitable for use.

In the course of these experiments, serums giving nonspecific fixability were occasionally included in the pool of complement for the next day's work, sometimes because the majority of serums tested were anticomplementary and had to be used; and sometimes to observe the effect in the pooled complement and on the antigen controls. After the tests were completed, the 1-10 dilutions were inactivated, and complement fixation tests carried out on them on the following day. Each day a sample of the pooled diluted complement was also inactivated, and a complement fixation test done on it.

Between April 27, 1931, and October 3, 1931, 400 guinea pig serums were tested. Of this number, 106 or one-quarter, showed nonspecific fixability with the Bordet antigen, but only 40 with the cholesterinized antigen. Approximately one-half (198) gave reactions in the Wassermann test with the Bordet antigen, but only 34 with the cholesterinized antigen. There were only 5 serums which showed nonspecific fixability with the Bordet antigen, which did not give reactions in the Wassermann test; but there were 98 serums which did not show nonspecific fixability in the preliminary test, which did give reactions in the Wassermann test.

The suggestion is made that the fact that the tests for nonspecific fixability are held for two hours at ice box temperature, while the complement fixation tests are held for four hours, may be of influence here. A 1-20 dilution of guinea pig serum for the test for nonspecific fixability is advised by the State Laboratory, and used there, and it is possible that the anticomplementary quality of some of the guinea pig serums was masked by the stronger hemolytic activity of the 1-10 dilution.

Kahn tests were done on 60 of the 106 serums which gave nonspecific fixability with Bordet's antigen. All Kahn tests were negative.

An examination of the records was made, to see whether the length of time required for hemolysis at 37° C. in the test for nonspecific fixability bore any relation to the positive Wassermann tests. Following are the figures for this: time required for hemolysis, ten minutes, 1 serum; nine minutes, 3 serums; eight minutes, 8 serums; seven minutes, 15 serums; six minutes, 17 serums; five minutes, 9 serums; four minutes, 19 serums; three minutes, 26 serums. There were as many serums that hemolyzed rapidly, giving reactions in the Wassermann test as serums that hemolyzed slowly.

The age, when tested, of the 1-10 dilution of guinea pig serums had no bearing on the positive results in the Wassermann tests. Sixty-five of the 98 serum dilu-

tions were twenty-four hours old when tested, while only 33 were forty-eight hours old.

These results show that neither the length of time required for complete hemolysis in the preliminary test, nor the age of the 1-10 serum dilutions when tested, were responsible for the positive reactions in the Wassermann test.

Records were kept of the 112 pooled diluted complements which were used during the period while the 400 serums were being tested. Of these 112 pools, 103 had samples inactivated, and Wassermann tests done. A table has been made showing the relation between the presence of a reaction with the Bordet antigen in the pooled complement and whether or not the Bordet antigen controls were anticomplementary.

Wassermann − : antigen controls negative	47	85.44% agreement
Wassermann + : antigen controls anticomplementary	41	
Wassermann − : antigen controls anticomplementary	15	
Wassermann + : antigen controls negative	0	
Total	103	

Of the 15 pooled complements having negative Wassermann tests and the Bordet antigen controls anticomplementary, 13 pools had included in them serums which gave reactions in the Wassermann test. Of the 56 pooled complements which had 0.1 c.c. Bordet antigen controls anticomplementary, only 21 had the 0.4 c.c. controls anticomplementary, also.

The 400 serums, the results on which have been presented, do not represent 400 guinea pigs, but instead, 400 consecutive bleedings. To determine the incidence of this type of reaction in guinea pigs used here, serums from 100 consecutive guinea pigs were tested. There were no duplicates in this series. Of the 100 guinea pig serums, 35 either showed nonspecific fixability with Bordet's antigen, or gave a reaction with Bordet's antigen in the complement fixation test. Sixteen of the 35 guinea pigs were males, and 19 were females.

There were 100 guinea pigs which had two or more bleedings. Of these, in 46, the serum was always satisfactory; in 17, it was always unsatisfactory with Bordet's antigen; and in 37, the results of the bleedings varied. Of the satisfactory 46, 21 were males and 25 were females; of the 17 unsatisfactory, 9 were males and 8 were females; of the 37 variables, 22 were males and 15 were females. These tests were carried on into January, 1932, in order to include in this paper the results on as many guinea pigs as possible.

Apparently the sex of the guinea pig is not responsible for the nonspecific fixability with Bordet's antigen, nor for its variability in the same guinea pig.

Twelve of these guinea pigs were examined for lesions suggestive of spirochetal infection of the genitalia. No pathologic conditions were seen. Secretions from the genitalia of these animals were examined by dark-field illumination. Coarse, thick spirochetes and smaller short spiral forms were found in specimens from 7 of the animals. None of the spirochetes resembled *Spirocheta cuniculi.*

A search of the literature revealed an article by V. Tomasek on this subject.[2] The author noted that some individual samples of guinea pig serum reacted nonspecifically with Bordet's antigen, but gave no reaction with Kolmer's antigen.

He found that cold weather, and lack of green food in winter, were contributing factors because the largest number of unstable complements were found at this time. But he thought the method of preparation of the antigen was the cause, and that it was probably due to the *preliminary* extraction of the beef heart with acetone, since plain alcoholic extracts, and the preliminary extraction with ether (as in Kolmer's antigen) do not result in nonspecific fixability. He thought it probable that the extraction with acetone changed the proportion of the lipoids of the extract, and that this quantitative change might be the cause of the anti-complementary power.

Tomasek thought it extremely important to test each guinea pig serum for anticomplementary properties; and not to pool such serums with those which reacted normally, because the mixture of poor and normal complement is sometimes fixed by Bordet's antigen in the same way that the weak complement itself is.

The tests carried out here were during late spring, summer and early fall, when cold weather was not a contributing factor.

This paper was presented before the meeting of the New York State Association of Public Health Laboratories in Albany on November 8, 1931. Discussion brought out the fact that at the State Laboratory where preliminary tests for nonspecific fixability with both antigens are done on all guinea pig serums to be used as complement, only a small number of serums react with Bordet's antigen. This does not confirm our results. Another laboratory in the state, which carries out a large number of Wassermann tests, but which does no preliminary tests on guinea pig serums, reported that it also found many spinal fluids positive with Bordet's antigen, but not with the cholesterinized antigen; this bears out our findings.

It was suggested that possibly the diet of the animals might be an important factor. The guinea pigs used here are fed, the year round, on a diet of cabbage, alfalfa, and oats. The alfalfa is cut from the grounds surrounding the animal house and in the summer it is fed to the animals green, but in the winter it is dried. Ninety per cent of the guinea pigs used here are purchased from dealers in Rochester and vicinity.

It was also suggested that the reason why serums were not affected like spinal fluids in the Wassermann tests was due to the buffer action of the serums.

No suggestion was offered as to why the 0.4 c.c. antigen controls often hemolyzed while the 0.1 c.c. controls did not.

Since this paper was presented, preliminary tests for nonspecific fixability with both antigens were carried out, using both 1-10 and 1-20 serum dilutions. One hundred and ninety consecutive serums were tested. With Bordet's antigen, 23 serums showed nonspecific fixability with the 1-20 dilution, and 19 with the 1-10 dilution. With the cholesterinized antigen, 25 showed nonspecific fixability with the 1-20 dilution, but only 11 with the 1-10 dilution. These figures show that the 1-20 dilution shows up many more cases of nonspecific fixability with the cholesterinized antigen than does the 1-10 dilution; but that with Bordet's antigen, the difference between the two dilutions is much less marked. In the 1-20 dilution about the same number of guinea pig serums reacted with each antigen; about one-half (13) reacted with both antigens.

It seems evident that it is better to use a 1-20, than a 1-10 dilution of guinea pig serum for tests for nonspecific fixability.

SUMMARY

The results presented in this paper show:

1. Guinea pig serum is more apt to show nonspecific fixability with Bordet's antigen than with the cholesterinized antigen (106 out of 400, as against 40).

2. Serums showing nonspecific fixability with Bordet's antigen will usually give reactions in the Wassermann test (only 5 out of 106 did not).

3. Many serums in a 1-10 dilution not showing nonspecific fixability with Bordet's antigen will give a reaction with the Wassermann test (98 out of 400 did). Some of these might have shown nonspecific fixability if tested in a 1-20 dilution.

4. These serums showing nonspecific fixability with Bordet's antigen give negative results with the Kahn test. (All 60 tested out of the 106 were negative.)

5. The length of time required for hemolysis in the test for nonspecific fixability, and the age of the 1-10 dilutions of guinea pig serum when tested, have no bearing on the occurrence of a reaction in the Wassermann test.

6. There was fairly good agreement (85.44 per cent) between the presence or absence of a reaction in the Wassermann test on the pooled diluted complement, and the presence or absence of anticomplementary action in the Bordet antigen controls.

7. The serums of 35 per cent of 100 different pigs bled and tested consecutively showed nonspecific fixability with Bordet's antigen, or gave reactions with Bordet's antigen in the complement fixation test, or both.

8. Of 100 guinea pigs which were bled two or more times, in 46 the serum was always negative with Bordet's antigen, in 17 it was always unsatisfactory, and in 37 variable.

9. The sex of the guinea pig did not seem to be a factor in the quality of nonspecific fixability with Bordet's antigen, or of its variability in the same guinea pig.

CONCLUSIONS

Since at least one-third of the guinea pig serums tested in this laboratory were not suitable for use with Bordet's antigen, and since the inclusion of unsuitable serums in the pooled complement gave rise to apparently nonspecific reactions with Bordet's antigen on spinal fluids, it is obvious that all guinea pig serums should be subjected to a preliminary test for nonspecific fixability with Bordet's antigen, and only those serums which do not react with this antigen should be included in the pooled complement.

REFERENCES

1. Wadsworth, Augustus B.: Standard Methods of the Division of Laboratories and Research of the New York State Department of Health, 1927, Baltimore, Williams and Wilkins Co.

2. Tomasek, V.: Sur le pouvoir antialexique de l'antigène de Bordet-Ruelens, Compt. rend. Soc. de Biol. **99**: 973-975, 1928.

THE SPECIFICITY OF THE KAHN TEST IN MALARIA*

CLAUDE R. SMITH, M.D., DETROIT, MICH.

THERE have been many reports in the literature of nonspecific Wassermann reactions in malaria. Weil and Braun[1] report false positives in malaria. Others using a wide variety of technic report false positive reactions, but Kolmer[2] states that "The sera in acute febrile diseases may become somewhat more anticomplementary than usual but with due care in technic these nonspecific positive Wassermann reactions can be avoided." The fact of reported false positive Wassermann reactions in malaria has perhaps led to an idea that positive serology in malaria patients is not trustworthy.

I am unable to find reports of false positive Kahn reactions in malaria, but, in regard to the febrile diseases, Jui-Wu-Mu and Keim[3] report a series of Kahn and Kolmer examinations in febrile diseases including pneumonia, typhus, relapsing and typhoid fever. They find that the Kahn reaction is not appreciably affected by the febrile reaction in these diseases. Besancon and Mayer[4] made a study of the Kahn reaction during chill and fever due to nonspecific protein. They state that the Kahn test shows a tendency toward negativity in both chill and fever.

The blood examinations reported in this communication were done in the hospital of the Cia, Ford Industriel do Brazil, situated in the Amazon River Valley, four degrees south of the equator. This is a highly malarious section and one in which the incidence of syphilis is known to be high. Yaws is also fairly prevalent. Maxwell[5] and others have shown that positive Kahn reactions are obtained from the blood of patients suffering from yaws. Badger[6] has shown that the incidence of positive Kahn tests in leprosy is high, and, since leprosy is very prevalent in the Amazon valley, this factor cannot be discounted. However, none of the patients in this series were suffering from either leprosy or yaws in a stage that could be recognized clinically.

The routine diagnostic test with serum as described by Kahn was done in these cases. The antigen was purchased from American biologic houses manufacturing it.[7] Readings were made in accordance with the instructions given by Kahn.[8]

The procedure was as follows:

Blood smears for malaria and blood for Kahn tests were obtained routinely from newly admitted patients. The malaria smears were stained with Wright's stain and examined immediately. The Kahn tests were done at intervals of from two to three days. The temperature recorded here is the admission temperature. Repeat examinations were done on those patients who could be located after three to six weeks.

The results of the tests fall into the following groups:

Positive malaria and negative Kahn	116
Positive malaria and positive ++++ Kahn	61
Positive malaria and positive +++ Kahn	6
Positive malaria and positive ++ Kahn	11
Total	194

*Received for publication, February 15, 1932.

The above figures show 60 per cent of the Kahn reactions negative, or that a preponderance of patients acutely sick of malaria have negative Kahn reactions.

On the other hand 40 per cent of the Kahn reactions are positive which is certainly a high percentage. However, the incidence of clinical syphilis is very high, numerous cases of yaws are seen and leprosy is widespread. The general incidence of positive Kahn reactions is 41 per cent, 452 positive in 1099 reactions. So that the incidence in this group of malaria patients is slightly less than the general incidence.

Of the positive Kahn reactions it was possible to repeat 45 after an interval varying from three to six weeks. At this time all were clinically well of malaria, without fever, and showed negative blood slides.

Results of repeat examinations (positive Kahns).—

Reaction same as first test	39
Increased from ++ to ++++	3
Increased from +++ to ++++	1
Decreased from ++++ to ++	2

The group of 4 patients whose reaction had increased had taken no treatment, while those 2 whose reaction had decreased had each taken 6 injections of neosalvarsan.

Eleven of those who had negative Kahn reactions and positive blood smears were repeated when free of malaria. Their Kahn tests all remained negative.

Relation of admission temperature to Kahn reactions.—

Of the 78 patients who showed both positive Kahn reactions and malaria on examination, the admission temperature was as follows:

Normal	37° C.	13
Subnormal	35°-37° C.	18
Abnormal	37°-40° C.	47

Of the 116 patients showing negative Kahn test and positive malaria, the admission temperature was as follows:

Normal	37° C.	16
Subnormal	35°-37° C.	31
Abnormal	37°-40° C.	69

These two tables show that essentially the same temperature reaction occurs in the group showing positive Kahn tests as in the group showing negative Kahn tests; 60 per cent of those with positive Kahn tests show fever, while 59 per cent of those with negative Kahn tests show fever. These tables also show that the febrile group of 116 patients yield 40 per cent positive Kahn reactions, and in the group showing no fever the incidence of positive Kahn reactions was also 40 per cent.

SUMMARY

Kahn tests were done on the blood of 194 patients who were acutely sick of malaria.

The incidence of positive Kahn tests is slightly less in this group than it is in all admissions to this hospital.

Repeat examinations of 56 patients were done and yielded essentially the same result as the first examination.

The preponderance of Kahn tests are negative.

Those having fever show the same incidence of positive Kahns as those not having fever. Both of these figures are approximately the same as the general incidence of positive Kahn tests.

CONCLUSIONS

The specificity of the Kahn test is not affected by acute malaria, or its febrile reaction.

REFERENCES

1. Weil, E., and Braun, H.: Uber die Entwicklung der Serodiagnostik bei Lues, Wien. klin. Wchnschr. **21**: 624, 650, 938, 1908.
2. Kolmer: The Specificity, Sensitiveness and Practical Value of the Kolmer Wassermann Reaction, Am. J. Syph. **18**: 248, 1929.
3. Jui-Wu-Mu and Keim: Arch. Dermat. & Syph. **17**: 387, 390, 1928.
4. Besancon, John H., and Mayer, Verna R.: The Sensitivity of the Kolmer and Kahn Tests During Chill and Fever, J. LAB. & CLIN. MED. **15**: 25-29, 1929.
5. Maxwell, J. S.: J. Trop. Med. **30**: 294-296, 1927.
6. U. S. Public Health Reports, **46**: No. 17, 1931.
7. Digestive Ferments Company and Parke-Davis & Company, Detroit, Mich.
8. Kahn, R. L.: Kahn Test a Practical Guide, 1928, Williams and Wilkins.

HENRY FORD HOSPITAL.

LABORATORY METHODS

A COLORIMETRIC METHOD FOR THE ESTIMATION OF UROBILINOGEN IN URINE AND FECES

L. D. SCOTT, LONDON, ENGLAND

THE reaction of Ehrlich[1] for the recognition of urobilinogen in urine is too well known to need but a casual reference since it was first described by him in 1901. While investigating the aniline dyes for their effects upon trypanosomes, Ehrlich noticed that the addition of paradimethylaminobenzaldehyde to certain fresh urines produced a bright red coloration.

Two years later Pappenheim[2] called attention to the fact that the reaction only occurred in those urines that, on standing, gave the reaction for urobilin. Later, Neubauer[3] demonstrated that the reaction was due to urobilinogen, a colorless chromogen which gradually becomes converted into urobilin by the action of light and oxygen.

Although after these many years rather strong criticisms of the reaction have appeared, the simplicity of the test has always insured for it a permanent, if not paramount place.

It was therefore decided to ascertain how far the reaction could be applied for a quantitative method. Investigation showed that it was capable of application to urine and feces, and that this quantitative process preserves the simplicity of the original reaction.

The limitations of this process arose from the fact that if the red coloration produced by the aldehyde salt from urobilinogen were to be compared against a standard solution (and it follows that for convenience the standard must be an artificial one), the yellow color of the urine would seriously interfere with the comparison. Also the yellow color of the reagent itself, as well as the yellowish green color produced by it from urea, would render comparison impossible. Then there are the other various practical points of difficulty that have been raised in the past which have to be considered. They may be divided into the following categories:

1. The reaction is not consistent with results, due presumably to the many variations in the technic recommended to bring about the reaction. This point alone emphasizes the need for a standard method.

2. Likewise, the ingestion of certain drugs such as hexamethylamine or antipyrine are known to produce urobilinogenuria.

3. The presence of acetone in the urine must also be excluded as it produces a similar coloration.

4. And again the effect of hydrochloric acid in excess on other urinary pigments produces a marked red coloration, hence an excess of the aldehyde salt must be avoided.

First, the problem resolved itself into finding a quantitative method employing the aldehyde salt of Ehrlich, and that the red color so produced from urobilinogen

under standard conditions, to be freed from the yellow color of the urine, and then compared against a standard solution.

Second, the presence of drugs in the urine do not cause a reaction characteristic of the protochrome, but, of course, the urobilinogenuria caused by the ingestion of drugs is not preventable.

Third, this special dilution employed prevents acetone and diacetic acid reacting to give the slightest red coloration.

Fourth, the amount of hydrochloric acid introduced with the benzaldehyde salt is such that no reaction can occur with the urinary pigments.

The method as presented here may be summarized as follows:

A measured amount of urine is treated with an appropriate amount of a specially prepared solution of paradimethylaminobenzaldehyde. After standing for a given time the mixture is heated under standard conditions in a boiling water-bath for a short period, and after cooling the coloration so produced is extracted with amyl or butyl alcohol. This extracted red coloration which is practically free from foreign material and other interfering colors, is then compared in the colorimeter against a standard ferric thiocyanate solution almost identical with that employed by Van den Bergh[4] for the estimation of bilirubin in blood.

The urines examined are from such cases where urobilinogen appears in the urine, i. e., with hepatic diseases, diabetes mellitus, tuberculosis, infectious diseases, visceral hemorrhage, and heart diseases in stage of decompensation.

Solutions:

1. A special dilution of Ehrlich's benzaldehyde salt. Paradimethylaminobenzaldehyde 10 gm. Pure concentrated HCL 100 c.c. Distilled water to 300 c.c. To about 100 c.c. of distilled water add the 100 c.c. of concentrated hydrochloric acid, mix and then add 10 gm. of paradimethylaminobenzaldehyde. Finally make the volume to 300 c.c. with distilled water.
2. A saturated aqueous solution of ammonium sulphate.
3. Pure amyl and butyl alcohol.
4. Special large thick walled test tubes (Monax combustion tubes 6 inches by ⅝ inch) graduated to hold about 25 c.c.
5. Standard ferric thiocyanate solution as modified by the author in a recent paper.

Stock Solution A.—Dissolve 0.1508 gm. of ammonium iron alum in 50 c.c. of concentrated hydrochloric acid, and then add water to 100 c.c.

For Stock Solution B.—Take 10 c.c. of Solution A, plus 25 c.c. of concentrated hydrochloric acid, and water to 250 c.c. This final solution is quite stable and keeps for several months. Then for the comparison standard take 3 c.c. of Solution B, plus 3 c.c. of 20 per cent potassium thiocyanate, mix well, and extract with 12 c.c. of amyl or butyl alcohol depending upon the alcohol used for the extraction of the unknown solution.

This standard corresponds to a concentration of 1 in 200,000 of azo-bilirubin, or 0.5 mg. (1 Van den Bergh unit).

As there seems no objection to this arbitrary standard both for quickness and simplicity it has been adopted for the aldehyde reaction, substituting urobilinogen for the above dilution of 1 in 200,000 of azo-bilirubin.

METHOD

To 10 c.c. of a fresh urine in one of the thick walled test tubes add 0.5 c.c. of Ehrlich's reagent. After standing for ten minutes at room temperature to allow

"coupling" to take place, immerse the tube in a beaker of boiling water for exactly one minute. Then cool immediately in running water. The coloration so produced is extracted by adding 1 c.c. of saturated solution of ammonium sulphate plus 10 c.c. of amyl or butyl alcohol, and shaking thoroughly for about one minute. Now allow the alcohol roughly to separate, after which it can be pipetted or decanted into a large centrifuge tube and then spun for a few minutes to separate the alcohol completely.

It would be as well to mention before proceeding that experience has shown the butyl alcohol to be superior to the amyl alcohol, in that it does not produce such a persistent emulsion with protein and such like interfering substances as does the amyl alcohol. It can also absorb a far greater amount of "azo-urobilinogen" which is to a distinct advantage when used for the feces process where strong colors have to be dealt with. The extracted reddish pink color produced from the urine is then compared against the iron rhodanate standard which should be of half strength (1.5 c.c. Solution B, 1.5 c.c. of 20 per cent potassium thiocyanate, 12 c.c. butyl alcohol), as this is most suitable for comparison with the majority of urines. A correction must, of course, be made in the final calculation to account for this.

As regards the unknown solution a characteristic reddish pink color is only obtained in those urines where the urobilinogen content is raised above the so-called "normal" limits, while with strictly normal urines the alcoholic extract is colored a faint brown tinge, which is quite incomparable against the ferric thiocyanate standard.

The calculation is as follows:

$$\frac{\text{Standard}}{\text{Unknown}} \times \frac{\text{Alcoholic extract in c.c. (10 c.c.)}}{\text{Amount of urine used (10 c.c.)}} \times \frac{\text{Strength of standard}}{\text{(0.5 or 0.25 mg., i. e. 1 or 0.5 unit)}}$$

equals units of urobilinogen per 100 c.c. of urine.

Table I shows some of the findings by this method:

TABLE I

TYPE OF CASE	UROBILINOGEN	
	IN UNITS	IN MG.
Thirty normal subjects	0 to 0.50	—
Three cases of diabetes mellitus	0.33	0.16
	0.44	0.22
	0.89	0.44
Heart disease	28.00	14.00
Two cases of pulmonary tuberculosis	0.23	0.11
	0.59	0.29
Pernicious anemia	2.50	1.25
Postoperative cholecystectomy	4.00	2.00
(A) Acholuric jaundice	2.53	1.26
Typhoid fever	3.55	1.77
First stages of catarrhal jaundice	7.85	3.92
(B) Hemolytic jaundice	5.20	2.60
(C) Cirrhosis of liver	10.55	5.27
(D) Anemia	1.85	0.92
(F) Splenic anemia	2.49	1.24
Pulmonary tuberculosis	1.61	0.80
Hemolytic jaundice	14.30	7.15

From the above results it will be observed that a reaction is only obtained if the urobilinogen content is over 0.20 unit and if below this amount, as previously stated, only a brownish-yellow tinge is extracted, and that the real abnormal urobilinogen content of the urine is above 0.50 unit, but that urines from apparently normal individuals may give a reaction which lies between 0.20 and 0.50 unit.

Before proceeding with the details of the analysis for the urobilinogen content of feces, it will be as well to refer to the slight difference in technic as that used for the urinary process.

First, a suspension of feces is made in water, and after thorough mixing to ensure an even emulsion, the aldehyde salt is added. The amount of this salt added is in excess of that used for the urine analysis, as some of the HCl in the reagent will be buffered by the fecal material. After the standard heating conditions, ammonium sulphate is added to the cooled solution, the addition of which not only aids in the separation of the solid material from the alcohol, but also causes complete extraction of the red coloration, by the butyl alcohol. After centrifuging, the clear red alcoholic layer is transferred to the colorimeter, and compared against the iron rhodanate standard.

Second, as the reddish pink color so produced is most pronounced with the majority of feces it is usual to make the comparison against a double or even a quadrupled standard.

Third, to prevent any pink coloration due to a reaction between indol in the feces and the benzaldehyde solution, the same dilutions are employed as those used for the urine method.

METHOD

Into one of the thick walled test tubes weigh out 0.5 gm. of wet feces. Suspend this in about 5 c.c. of water by inserting a rubber stopper and shaking violently to break up any hard solid material that may be present. To the fecal solution is now added 3 c.c. of Ehrlich's reagent and after diluting the volume to 10 c.c. with water, again mix well. After standing at room temperature for ten minutes, immerse the tube in a beaker of boiling water for one minute, and then cool. Two cubic centimeters of saturated solution of ammonium sulphate are added followed by 10 c.c. of butyl alcohol. Now mix thoroughly, and the alcohol is then allowed to separate roughly as specified for the urinary process. Pipette or decanter the supernatant material into a centrifuge tube and then spin for five minutes or so, or until the alcoholic extract is clear. If the color of the unknown solution is still too deep even for the quadrupled standard, it may be diluted with a further addition of butyl alcohol, but for most cases it will be found that the double standard (6 c.c. of Solution B, 6 c.c. of 20 per cent potassium thiocyanate, and 12 c.c. of butyl alcohol) is most convenient.

Finally it must be remembered that for the urine or the feces process the operator must himself judge the most suitable standard for comparison with the unknown solution, and also that this comparison must always be calculated as for the 1 unit standard (0.5 mg.).

A small quantity of wet feces, about one gram, is then dried in a small evaporation basin to constant weight, and the necessary correction is made in the final calculation to allow for the water percentage in the feces examined.

The calculation is as follows:

$$\frac{\text{Standard}}{\text{Unknown}} \times \frac{\text{Dilution of (10 c.c.) color in c.c.}}{\text{Amount of wet feces taken (0.5 gm.)}} \times \frac{\text{Strength of standard}}{\text{(1, 2, 3, 4 units, i. e. 0.5, 1, 1.5, 2 mg.)}}$$

equals units of urobilinogen per 100 gm. of wet feces.

The following form contains the directions for the correction of the water percentage of the feces:

Exactly one gram of wet feces is weighed out into a small porcelain dish or crucible and dried to constant weight by means of a boiling water-bath and a vacuum dessiccator. Suppose for example the weight of the dish plus one gram of wet feces is 18.8060 gm., the weight of the dish alone being 17.8060 gm. After drying, the weight is 18.1936 gm. Therefore, the weight of dry material in 1 gm. of wet feces amounts to 0.3876 gm. This figure multiplied by 100 gives the percentage of solid contained in the sample of wet feces. In the example quoted it will be 38.76 per cent.

As the original equation expresses the urobilinogen content in units per 100 gm. of wet feces, the result obtained multiplied by

$$\frac{100}{\text{amt. of solid material expressed in percentage}}$$

will correct the urobilinogen content for 100 gm. of dried feces.

The above illustration, as well as the final equation, are selected from Case 1 in Table II given below. The wet feces contained 92.3 units urobilinogen per 100 gm. The solid material was 38.76 per cent. Therefore, 100 gm. of dry feces will contain

$$\frac{100}{92.3 \times 38.76} = 238.1 \text{ units.}$$

Table II epitomises the results obtained by this method of analysis.

Before summarizing the above results, it would be interesting to give some notes of the individual cases examined.

In the urinary analysis of Case A, no bile pigments or salts could be detected, but the Van den Bergh reaction in the blood gave a delayed direct reaction: the indirect test showing 5.5 units of bilirubin.

Case B.—Bile absent in the urine, but the reaction for bile salts was strongly positive. The blood gave a delayed direct reaction with a content of 3.3 units of bile.

Case C.—No bile pigments or salts present in urine. The direct reactions for bile in the blood were negative, but the indirect reaction gave 0.81 units of bilirubin.

Case D.—The urine gave a practically normal content of urobilinogen, but the feces contained a large amount of the chromogen which would be expected in this type of case.

In Case E, the results of the urine, blood, and feces examinations are most interesting. The urine contained 15 units of bilirubin, while the bile salt content was also large. The urobilinogen reaction was, of course, negative, as would be expected. The diastase content was 60 units. The blood gave a strong direct reaction for bile, the indirect reaction content being 21 units. The feces gave a completely negative reaction for urobilinogen, proving that the chromogen is decreased or entirely absent in cases of obstructive jaundice.

Case F.—Urine contained no bile pigments or salts. The blood gave negative direct reaction for bilirubin, but a positive indirect reaction corresponding

TABLE II

TYPE OF CASE	COLOR AND NATURE OF FECES	UROBILINOGEN CONTENT		
		UNITS	CORRECTED FOR WATER PERCENTAGE	
			UNITS	MG.
Normal feces 1	In all six cases color normal, light brown. Percentage of solid material 38.76%	92.3	238.1	119.0
2	23.57%	82.2	348.6	174.3
3	30.01%	61.7	205.5	102.7
4	34.60%	97.7	282.3	141.1
5	21.73%	74.8	344.2	172.1
6	42.47%	130.0	306.0	153.0
Pancreatic insufficiency	Very pale yellow. Fat content 57.51%. Solid material present in 28.01%	27.3	97.4	48.7
(D) Anemia	Dark brown. 28.98% solid material	120.0	414.0	207.0
Hemolytic jaundice	Dark dry feces. Solid material 31.84%	166.6	532.2	261.6
Pernicious anemia	Dark brown. Solid material 32.30%	171.1	529.7	264.8
Cholangitis	Color, brown. Solid matter 24.8%	103.0	415.3	207.6
Gross diarrhea	Solid matter 8.84%	67.5	763.5	381.7
Gastroenterostomy	Very pale yellow. Gave very strong mercuric chloride (Schmidt's) reaction for bile pigments. Unaltered bilirubin present to extent of 133.2 units on V. den Bergh scale. Solid material 18.01%	50.0	277.6	138.8
(E) Carcinoma of head of pancreas (causing obstructive jaundice)	Very light grey. Solid matter, 44.057%. Fat content, 41.73%		Urobilinogen completely negative	
Anemia	Dark brown. Solid material 19.26%	87.0	452.0	226.0
(F) Splenic anemia	Dark brown feces. Solid percentage 21.17%	141.3	667.4	333.7
Catarrhal jaundice	Very pale yellow. Solid percentage 52.35%	87.0	166.2	83.1
Five cases of obstructive jaundice	Pale grey		Completely negative	

to 3.2 units. The result of the feces examination for urobilinogen is recorded in the latter tabulation.

Lastly, the results from the feces process may be briefly concluded as follows:

The urobilinogen content of normal feces is much too variable to obtain any normal limits, but it will be observed from Table II that it is much lower than that obtained from such cases as anemia, pernicious anemia, and hemolytic jaundice, where the urobilinogen content is high.

Again with many diseases of the liver such as partial obstruction of the bile passages or complete obstruction, the chromogen content is certainly decreased in the former, and completely absent or mere traces present in the latter disease.

In conclusion, just two points of practical importance may be mentioned, these being that the urine and feces samples should be quite fresh and examined as soon as possible, otherwise paradoxical results will be obtained. Also the reddish pink coloration produced from urobilinogen should, if possible, be compared in the colorimeter at once, as fading may occur.

SUMMARY

1. Use is made of a simple and ready reaction for the detection of urobilinogen in urine. This reaction of Ehrlich is extremely delicate, while the reddish pink coloration produced from the chromogen in urine and feces with paradimethylaminobenzaldehyde, can be matched against a standard solution thus enabling an estimation to be made.

2. The quantitative process may enable a positive diagnosis to be made as to whether the amount of urobilinogen is abnormal or not. For instance, in such cases as pernicious anemia or hemolytic jaundice, there is a marked secretion of urobilinogen in the urine and a raised amount in the feces, whereas in cases of partial or complete obstruction of the liver, the chromogen content of the feces is decreased or entirely absent.

3. The test is of great practical value for the detection of "latent" jaundice when there is a marked secretion of urobilinogen. In this respect it is more delicate than the Van den Bergh reaction.

4. Various alterations in the original reaction have improved the technic and enabled an estimation of the chromogen to be made.

REFERENCES

1. Ehrlich: Centralbl. f. klin. med. **8**: 593, 1886.
2. Pappenheim: Berl. klin. Wchnschr. **2**: 42, 1903.
3. Neubauer: Sitz. d. Gesell. f. Morph. u. Physiol. **2**: 1903.
4. Hijmans van den Bergh: Der Gallenfarbstoff in Blute, 1918, Leiden.

12 BRAMFIELD ROAD, WANDSWORTH COMMON.

SURGICAL MAGGOTS IN THE TREATMENT OF INFECTED WOUNDS; CULTURE OF STERILE MAGGOTS*

WILLIAM ROBINSON, PH.D., WASHINGTON, D. C.

ATTENTION has been directed in the last three years to the beneficial effects produced by blowfly larvae in the treatment of chronic osteomyelitis and other infected wounds. Since the publication in 1929 and 1930 of Dr. Wm. S. Baer's remarkable results,[1, 2] from the use of maggots in osteomyelitis, his method has been employed with success in a number of hospitals in the United States and Canada. There have also recently appeared in the medical literature several articles (listed at the end of this paper) dealing with various aspects of culture and treatment and describing results obtained.

This treatment necessitates the production of large numbers of sterile maggots throughout the year, and presents a problem requiring detailed knowledge of a special nature. It is possible for hospitals to purchase sterile maggots ready for use, but often it is more satisfactory to rear them as needed. The present contribution describes in detail cultural methods which are being used with good results in this laboratory; and also discusses the necessary sterilizing technic. The green-bottle blowfly (Lucilia sericata Meigen), described in this article, is a satisfactory species for cultural purposes and for use in wounds.

ESSENTIAL FEATURES OF LIFE HISTORY AND HABITS OF THE BLOWFLY

The Egg.—Eggs are minute, white, and elongate, and are laid in clusters upon meat. They are soft and easily crushed, and should be handled carefully. Hatching occurs at incubator temperatures within twenty-four hours.

The Larva or Maggot.—The maggots which emerge from hatched eggs require access to food; otherwise they would perish. For ordinary rearing purposes, therefore, it is best to allow the eggs to remain upon the meat where the flies have laid them. This meat keeps the eggs moist and serves as food for the maggots. For the production of sterile maggots a special food is prepared and will be described later.

The maggot is pointed at the head end and has no eyes. It feeds upon meat and takes up its food chiefly in liquid form. Breathing takes place mainly through two spiracles in the posterior end. Maggots develop quickly and in five to seven days become full grown. They then cease feeding and wander for a time in search of a secluded place in which to pupate. A layer of sand provides a suitable environment for pupation.

The Pupa.—After it is quiescent the maggot becomes oval in shape and brownish in color. Transformation from the maggot to the fly then takes place and occupies usually about seven days. There is no feeding in the pupal stage.

*From the Division of Insects Affecting Man and Animals, Bureau of Entomology, United States Department of Agriculture.
Received for publication, May 31, 1932.

In the prepupal stage further development may be delayed for several weeks, if desired, by placing the insects in cold storage at a temperature of 4.5° to 7.0° C. (40° to 45° F.). Normal development may be induced by replacing them in a temperature of 26.5° C. (80° F.).

The Adult or Fly.—After the adult emerges from the pupal case it soon commences to feed. Flies have been found by Baer[3] to thrive well upon a mixture of 70 c.c. of water and 30 c.c. of honey, to which is added 3 gm. (about one-quarter cake) of yeast. A pad of absorbent cotton about 2 inches square is placed in a Petri dish and thoroughly moistened with the mixture. Granulated sugar, as used by Miller,[13] also appears to give good results. With sugar, a water-fountain dish, as described by Murdoch and Smart[14] or Miller should be added. In another dish is placed a piece of lean beef about 2 inches square for the flies to lay eggs upon.

Females commence to lay eggs about five days after emergence. The number laid daily by one fly varies considerably, but averages about 100. The egg-laying period may extend over three to five weeks, but a colony should be destroyed when egg production falls below a satisfactory level.

Temperature and Humidity Requirements.—All stages from the egg to adult appear to be adaptable to a fairly wide range of temperature—from 24° C. (75° F.) to as high as 35° C. (95° F.) in summer. It is best, however, to use a constant temperature in order to control the rate of growth and activity. A temperature of 26.5° C. (80° F.) has been found satisfactory. The degree of humidity in the air is of considerable importance, and here again a wide range is possible, from 40 to about 80 per cent relative humidity being tolerated but 55 to 65 per cent giving good results.

Continuous Breeding.—The life cycle from egg to death of the female is about forty-five to fifty days. Under favorable conditions in the laboratory, it is possible to have continuous breeding throughout the year. To keep the production of maggots unbroken it is best to reserve some of the eggs laid early in the life of the female and allow them to develop into another generation when it is necessary to replace the old brood. Under the treatment recommended here, there will probably be no trouble in producing succeeding generations in winter. Inbreeding is apparently not detrimental to egg production, but it may be advisable to mix with a strain from another laboratory every few months.

GENERAL METHODS OF REARING

The Rearing Cabinet.—For the successful production of maggots and flies throughout the year an incubator or rearing cabinet is practically essential. Such a cabinet can be made at comparatively low cost. It may be constructed of wood, with two glass doors in front opening outward and glass in each end. A shelf allows two layers of cages to be used. The inside dimensions of the cabinet will depend upon the number and size of the cages.

The heat required for the cabinet may be supplied by electric-light bulbs or coils of resistance wire, placed at one end of a compartment below the cabinet. The heaters are connected to a heat regulator in the cabinet. Regulators of various kinds are listed in catalogs of scientific apparatus.

At the other end of the lower compartment a humidity chamber is constructed. There are several devices available for this purpose. Satisfactory results can be obtained with a shallow, broad water pan designed to allow lengths of absorbent cloth to hang down into the water. An electric fan placed between the heaters and the water pan draws air through an opening in one end of the compartment over the heaters and blows the warmed air across the moist surface of the cloths. The air then goes upward through openings in the floor of the cabinet, and after circulating leaves the cabinet through small openings in the sides near the top. The degree of humidity can be varied with the extent of moist cloth and with the temperature of the water in the pan.

Disagreeable odors should not occur in the production of sterile maggots for use in wounds, but in rearing maggots through to the pupal stage considerable unpleasant odor will develop. It is best, therefore, to use a separate cabinet for this purpose and to draw off the odors through an exhaust fan at the top which is attached to an outside vent.

The Fly Cage.—Several types and sizes of cages have been used for the adults. The small, cylindrical, cloth-covered cage described by Simmons[17] has been found most satisfactory here. It consists of a circular base and top of wood, the top having a large circular opening and braced beneath to prevent cracking. The sides are metal rods threaded at top and bottom and held in place with two nuts at each end. The dimensions of the cage are 14 inches high and 10 inches in diameter. A cloth sack of mercerized lawn is made to be pulled over the cage and fastened at top and bottom with a cord in a slot cut around the edges. The sack is made with a short sleeve which provides access to the cage. The top is covered with bronze wire gauze. The cloth cover becomes soiled in a few days and should be replaced, and the cage also requires cleaning occasionally. Flies can be easily transferred to a clean cage by inverting the clean cage over the soiled one and removing the intervening gauze. Transference is hastened by darkening the lower cage.

The Maggot-Rearing Container.—Larvae are reared best in glass containers, and various shapes and sizes have been used. In rearing through to the adult stage excellent results have been obtained here with 600 c.c. beakers covered with two layers of cheesecloth. In the production of sterile larvae for use in wounds a special type of container is recommended and is described later. Larvae to be reared to the fly stage require a suitable place to pupate. A satisfactory method is to use a pan with about three-quarters inch of sterilized sand in the bottom. When the maggots become nearly full grown, the container, with top removed, is placed in the middle of the pan. The maggots will leave the container and go into the sand to pupate.

Feeding Technic.—Flies: The food dishes, previously described, are placed in the cage through the sleeve. When honey-yeast is used, the food-pad tends to dry out and should either be replaced daily or well moistened with water on the second and third days and replaced on the fourth day. The meat also dries and should be replaced daily.

Maggots: For stock rearing, sufficient meat is placed in the beaker to last the larval period. Little further attention is required until just before wandering

commences, when the beaker should be placed in the pan. Food for sterile maggots is described later.

Removal and Care of Eggs.—The eggs are deposited upon the meat, usually on the lower surface, in the fly cages. Meat having egg clusters should be removed in the Petri dishes from the cages at the end of each day and the dishes covered to prevent drying out. They should then be placed in the refrigerator to prevent hatching during the night. No meat should be left in the cages overnight because of the short hatching period of the eggs. In the morning most of the eggs are removed with a scalpel from the meat, and transferred to water for the sterilizing process. The meat with the remainder of the eggs may be placed in larva beakers to develop into stock flies.

Procedure in Establishing Colonies.—1. The necessary incubators should be constructed and thoroughly tested.

2. Fly cages and other equipment should be ready for use.

3. About 100 to 150 pupae should be placed in each cage and put in the incubator. Upon request, the Bureau of Entomology in Washington will supply a sufficient quantity of pupae of the species Lucilia sericata to start two or three colonies.

4. The food for adults should be placed in each cage as soon as emergence occurs and the meat should be added two or three days later. The flies will begin to lay eggs in five to ten days.

5. To build up sufficient colonies for the required egg production, a large number of maggots should be allowed to develop into flies before an attempt is made to use eggs for sterile maggots.

THE PRODUCTION OF STERILE MAGGOTS

Maggots for use in wounds are reared under sterile conditions. On account of the possibility of serious infection from this source, it is strongly advised that the strictest bacteriologic technic be employed in this work. Sterilization is done in the egg stage, and the maggots are kept from contamination by the use of sterilized containers and food. Aseptic technic must be practiced throughout the entire process.

The eggs, which are usually laid in clumps, must be separated before sterilization is attempted. Baer[3] suggests vigorous stirring in water, Murdoch and Smart[14] use Dakin's solution, and Child and Roberts[4] use sodium hypochlorite solution.

Various methods and formulae have been recommended for sterilizing the eggs.[3, 4, 14, 21] White[21] has obtained favorable results with a solution consisting of mercuric chloride 0.25 gm.; sodium chloride, 6.5 gm.; hydrochloric acid, 1.25 c.c.; ethyl alcohol, 250 c.c.; distilled water, 750 c.c. The eggs are immersed for fifteen minutes. The formulae used by Murdoch and Smart and also by Baer are somewhat similar to that of White. Child and Roberts have used a three-minute immersion in a "solution containing 4 per cent formaldehyde." White has also used formaldehyde and states that a 5 per cent solution (of formalin) gives good results.

After sterilization the eggs must be thoroughly washed. A method suggested

by both Baer and White has been found convenient. It consists in transferring the eggs to cheesecloth in a Gooch crucible supported by a wide-mouthed bottle, and washing several times with sterile water.

The next step is to remove the eggs to the larval food vial. This can be quickly done by placing the cheesecloth containing the eggs directly in the vial.

Convenient food containers can be made from shell vials about 80 mm. high and 30 mm. wide or wide-mouthed bottles of similar size. The food may consist of pieces of liver in nutrient agar and should be sterilized in an autoclave. Food to the depth of 10 mm. is sufficient to carry the larvae over to the stage at which they are to be used in the wound. After the eggs hatch the gauze is removed from the vial.

On the second day after hatching, each lot of maggots is tested for sterility, with respect to both aerobes and anaerobes. Cultures are obtained from the partially liquefied food upon which the maggots are feeding. The maggots are then placed in the refrigerator to prevent development while the cultures are being incubated. Any vial of maggots which later shows contamination is discarded. At the completion of the sterility tests the maggots are still quite small, from 5 to 8 mm. in length. The small size is best suited for use in the wound, as the maggots feed for only a few days at the most.

ENTOMOLOGIC ASPECTS OF THE MAGGOT TREATMENT

Implantation of Maggots.—Sterile maggots are placed in the wound two to five days after the operation to remove devitalized bone has been performed. Baer,[3] Myers and Czaja,[15] Child and Roberts,[4] and Hewitt[10] describe the surgical procedure in cases for maggot treatment; and there is an abundant literature dealing with routine procedure in the operative treatment of osteomyelitis. Several methods have been used in transferring the maggots from the container to the wound. A simple method developed by White is to pour saline into the larval food vial, strain the maggots with gauze in the same way as was done with the sterilized eggs, and then transfer the larvae from the gauze directly to the wound. Aseptic technic must, of course, be used. Maggots received in vials free from food and ready for use may be removed conveniently with a cotton-tipped wood applicator.

The number of maggots used will depend upon the size of the wound, but will probably be from 200 to 600. Overcrowding should be carefully avoided. The maggots grow rapidly after implantation, and if too many are used they will shortly crowd each other and force some to leave the wound and crawl over the skin and get in the bed. It is best to use only as many as can be accommodated in the wound when they are full grown.

The Use of Cages Over the Wound.—To confine the maggots within the wound, cages of several kinds have been used. Hewitt, however, has obtained satisfactory results with no cage at all in certain cases. When first placed in the wound, some of the maggots will tend to wander. Hewitt found that if the wound is fanned briskly the maggots will be cooled by evaporation and go into the wound for warmth. They may also be pushed back with an applicator. The patient himself can do this. Any persistent wanderers may be removed. Feed-

ing will soon begin and the maggots will arrange themselves side by side with heads downward.

No Danger of Parasitism.—The maggots become full grown in from four to five days after implantation. They then cease feeding and are of no further use in the wound. In nature the maggots leave their feeding place and migrate until they reach a suitably dry and dark place for pupation. Therefore, with the species Lucilia sericata recommended and supplied by the Bureau of Entomology, there is no danger of parasitism within the patient or of injury to the tissues through neglect to remove the maggots. However, as pointed out in a circular issued by the Bureau of Entomology,[16] there is grave danger in the use of the screw worm (Cochliomyia macellaria Fab.) and that species must be carefully avoided. Before the end of the feeding period the maggots should be removed; otherwise they will leave the wound when feeding is finished and cause considerable annoyance to the patient.

The attitude of patients to the use of maggots is usually one of tolerance and cooperation. During the early stages of treatment, the maggots sometimes cause sharp, intermittent pain within the wound. Also an abundant drainage from the wound occasionally irritates the skin, and a brief rise in temperature may occur. These and other medical aspects of the treatment are discussed in the articles already referred to.

Number of Implantations.—After each removal of the full grown larvae from the wound, the patient may be given a day to rest. After several implantations, the irritating discharge usually changes to a copious serous discharge which does not inflame the surrounding skin. The number of implantations will vary with the individual cases, but are usually continued for six to eight weeks.

REFERENCES

1. Baer, W. S.: Viable Antiseptic in Chronic Osteomyelitis, Proc. Interstate Postgraduate Medical Assembly of North America, Detroit, Mich., p. 370-372, 1929.
2. Baer, W. S.: The Treatment and Cure of the Disease Known as Osteomyelitis. Hearings before Committee on World War Veterans' Legislation; House of Representatives, 71st Congress, 2nd Session, Washington, D. C., 1930.
3. Baer, W. S.: The Treatment of Chronic Osteomyelitis With the Maggot (Larva of the Blowfly), J. Bone Joint Surg. **13**: 438-475, 1931.
4. Child, F. S., and Roberts, E. F.: The Treatment of Chronic Osteomyelitis With Live Maggots, N. Y. State J. Med. **31**: 937-943, 1931.
5. Drinkard, R. U.: Osteomyelitis, West Va. Med. J. **28**: 1, 1932.
6. Goldstein, H. I.: Maggots in the Treatment of Wound and Bone Infections, J. Bone Joint Surg. **13**: 476-478, 1931.
7. Goldstein, H. I.: Sterile Live Maggots in the Treatment of Osteomyelitis, Med. Rev. Rev. **37**: 361-365, 1931.
8. Hall, E. S.: The Use of Maggots in the Treatment of Wounds, Maine Med. J. **23**: 80-85, 1932.
9. Hegner, C. F.: Maggots in the Treatment of Chronic Osteomyelitis, Colo. Med. **28**: 286-287, 1931.
10. Hewitt, J. F.: Osteomyelitis, Development of the Use of Maggots in Treatment, Am. J. Nursing **32**: 31-40, 1932.
11. Livingstone, S. K.: Maggots in the Treatment of Chronic Osteomyelitis, Infected Wounds, and Compound Fractures, Surg. Gynec. Obst. **54**: 702-706, 1932.
12. Livingstone, S. K., and Prince, L. H.: The Treatment of Chronic Osteomyelitis With Special Reference to the Use of the Maggot Active Principle, J. A. M. A. **98**: 1143-1149, 1932.
13. Miller, D. F., Doan, C. A., and Wilson, E. H.: The Treatment of Osteomyelitis (Infection of Bone) With Fly Larvae, Ohio J. Sc. **32**: 1-9, 1932.
14. Murdoch, F. F., and Smart, T. L.: A Method of Producing Sterile Blowfly Larvae for Surgical Use, U. S. Naval Med. Bull. **29**: 406-417, 1931.

15. Myers, J., and Czaja, L. M.: The Maggot Treatment of Osteomyelitis, Ill. Med. J. **60**: 124-133, 1931.
16. Robinson, W.: The Use of Blowfly Maggots in the Treatment of Osteomyelitis and Certain Other Diseases, U. S. Dept. Agr. Bur. Ent. Multi. Circ. E-295, 1932.
17. Simmons, S. W.: The Use of Blowfly Maggots in the Treatment of Osteomyelitis: a Convenient Blowfly Cage, J. Econ. Entomol. **25**: (5), 1932.
18. Smyth, T. L.: Maggots Following Electro-Coagulation Treatment of Cancer, Am. Med. **37**: (10) 9 of Spec. Supp., 1931.
19. Weil, G. C., Nettrour, S., and Rohm, R.: Treatment of Acute Hematogenous Osteomyelitis, With Especial Reference to the Use of Maggots, Penn. M. J. **34**: 313-316, 1931.
20. Weil, G. C., Henry, J. P., Nettrour, S., and Sweadner, R.: The Cultivation and Sterilization of the Fly Larva or Maggot, West Va. Med. J. **27**: 458-461, 1931.
21. White, G. F.: Production of Sterile Maggots for Surgical Use, Presented Before Am. Soc. Parasitol., New Orleans, La., December 29 to 31, 1931, Abstract in J. Parasitol. **18**: 133, 1931.
22. Wilson, E. H., Doan, C. A., and Miller, D. F.: The Baer Maggot Treatment of Osteomyelitis. Preliminary Report of Twenty-Six Cases, J. A. M. A. **98**: 1149-1152, 1932.

EMBEDDING CURETTINGS FOR FROZEN SECTION*

RUDOLPH J. SHAFER, M.D., CORNING, NEW YORK

THE demand for a rapid diagnosis on surgical specimens has made the frozen section method decidedly popular with surgeons. Curettings from the uterus are frequently so small that it is difficult for the pathologist to make a frozen section. It is essential, however, to make a diagnosis as soon as possible. The usual methods of embedding in paraffin or celloidin require too much time and in most cases cannot be used.

The method which I have used for a number of years and which has proved satisfactory in my hands is as follows:

The fragments of tissue are spread out in a shallow dish (stender), covered with 10 per cent melted gelatin and allowed to harden. (This may be hastened by placing in the refrigerator.) As soon as the gelatin is hard the dish is placed in a jar containing a few drops of 40 per cent formaldehyde where it is allowed to remain about twelve hours for fixation of the tissue. It is then ready to be frozen and cut. The cut sections are placed in cold distilled water, floated on a slide, which has been smeared with egg-albumen, the excess water is drained off and the section covered with a cigarette paper and blotted. By carefully removing the cigarette paper the section may be run through any staining method desired.

The advantage of this method is that the smallest fragments may be examined easily and quickly.

*Received for publication, April 2, 1932.

ROUTINE BACTERIOLOGIC DIAGNOSIS OF DIPHTHERIA SWABS BY MEANS OF CLAUBERG'S BLOOD TELLURITE MEDIUM*

EVELYN S. LEWIS, M.A., BERKELEY, CALIF.

SINCE the introduction of Loeffler's serum[1] as a medium for the bacteriologic diagnosis of diphtheria, there have been many attempts to perfect a differential medium which might be worthy of substitution. The fact that salts of tellurium may exert a differential antiseptic action on various organisms has been known for over twenty years. Potassium tellurite and tellurates have been used especially in connection with various differential media for the bacteriologic diagnosis of diphtheria since it has been found that C. diphtheriae, and the diphtheroids in general, resist given concentrations of tellurites and tellurates more than the staphylococcus and colon typhoid group. The composition of the many tellurite media proposed for differential purposes has involved essentially only a difference in the enriching factors, the amount of tellurium salt being approximately the same in each case.

Among the earliest media proposed, which contained for their enriching factors serum and peptone water, were those of Conradi and Troch,[2] and Smith.[3] The former consisted of 0.02 per cent calcium tellurate with calcium bimolate in a medium very similar to that of Loeffler. The medium proposed by Smith differed from this in that approximately 0.01 per cent potassium tellurite was added to the horse serum peptone water agar. The main disadvantage to both these media lay in the fact that the growth of streptococci was entirely unrestricted. Pergola[4] devised a liquid transparent medium also using serum but with the addition of egg yolks for further nutritional purposes. An improvement over plain serum for enrichment was proposed by Douglas.[5] His tellurite medium contained sterile trypsinized serum which was considered an improvement over plain serum in that it was found that complete or partial neutralization of antitryptic power increased the value of serum for cultural purposes. This was further improved upon by Allison and Ayling[6] who added 0.05 per cent copper sulphate to the usual tellurite agar medium. By this means staphylococci, streptococci, pneumococci, N. catarrhalis, and B. proteus were ruled out and the differential characteristics of C. diphtheriae, C. hoffmanni, and C. xerosis were more accentuated. The copper sulphate apparently had no effect on the virulence of the diphtheria organisms. Finally whole blood was successfully substituted for serum in the usual tellurite media. In 1929 Clauberg[7] offered his cooked blood-ascitic fluid tellurite medium and in 1931[8] modified it to a simple medium containing equal volumes of blood-glycerine, tellurite, agar and laked sheep's blood. Anderson, Happold, McLeod and Thomson[9] also proposed a chocolate tellurite medium containing sterile meat infusion which had never been heated over 75° C. Differentiation by this medium was considered more successful than by Loeffler's serum slants. Using a blood-cystine enriching

*From the Department of Bacteriology, University of California.
Received for publication, May 11, 1932.

base for tellurite agar Pesch and Krämer[10] reported excellent differential results after the addition of copper sulphate.

However, of all the tellurite media proposed, that of Clauberg[7] has been most favorably commented upon. Among the many who have reported success with it have been Manzini,[11] Tempe,[12] Seistrop,[13] and Tallo.[14] Up to the present time results of actual use of Clauberg's modified medium[8] have not been reported in detail. Mention of its existence and purported success was made in the report of Anderson and his colleagues but details of its actual adoption are still lacking.

The simplicity of the medium and the success reported by Clauberg prompted the following test of its practicability for the routine testing of diphtheria swabs as compared to the usual staining of growth from Loeffler's serum slants.

THE MEDIUM

The medium was made precisely according to the directions given by Clauberg in his report of 1931.[8] Care was taken to use fresh ingredients only. The formula was as follows:

A. Blood mixture.
   1. One part sterile blood (fresh sheep or beef).
   2. Two parts sterile distilled water.

B. Blood-glycerine agar.
   1. Two parts sterile sheep blood.
   2. One part sterile glycerine.
   3. After six to eight weeks or more, mix aseptically the glycerine-blood in 5 per cent concentration with 3 per cent nutrient agar P_H 7.5 at a temperature between 45° and 50° C.

Add equal parts of mixture A and freshly made still fluid mixture B, plus four parts in a hundred of 1 per cent stock potassium tellurite. Pour the plates not over 5 mm. thick. Make fresh tellurite solution for each batch of medium. Eimer and Amend, C. P. potassium tellurite was found to dissolve readily upon warming the solution and further steps were never found necessary for its preparation. It was found advisable to prepare enough plates for not more than one week's supply, the stock being kept in the refrigerator until used.

The actual expense involved in making each type of medium could not be calculated for it would depend to a great extent upon the facilities of the laboratory. However, the cost of the basic ingredients* might be considered standard everywhere. Both the beef serum obtained from the slaughter house and the sterile sheep blood could always be obtained without cost.

The comparative expense connected with the production of Loeffler's and Clauberg's mediums was found to be practically the same, the only difference being in the amount of labor and in the number of plates and slants which could be produced from an equal volume of each type of medium. One hundred cubic centimeter volumes produced 50 to 100 Loeffler slants and 12 to 24 Clauberg plates depending upon the size of the test tubes and Petri dishes used.

---

*Potassium phosphate (Dibasic), C. P., Baker's Analyzed.
Potassium tellurite, Eimer and Amend.
Glycerine purified (U. S. P. X.)
NaCl, C. P., Baker's Analyzed.
Dextrose, Merck.
Peptone, (Bacto) Difco.
Beef extract, Liebig's.

Substitution of sterile rabbit blood was found to be unsatisfactory since the C. diphtheriae grew with noticeably less luxuriance than when sheep blood was used.

METHOD OF PROCEDURE

Through the kindness of Dr. W. H. Kellogg, Director of the State Bacteriological Laboratory of California, fresh swabs were available for the routine tests. Each swab was first smeared directly on Loeffler's serum slants then streaked on a Clauberg plate. All plates were examined after twenty-two to twenty-six hours' incubation at 37° C. The diagnosis of the plates was made solely by eye from the macroscopic appearance of the colonies in strong daylight. These results were compared with those obtained by staining a smear of the swab planted simultaneously on a Loeffler slant. In cases where the results differed or were doubtful the virulence of the strain was determined by the method proposed by Force and Beattie[15] for the routine testing of field cultures from suspected diphtheria cases. Also the fermentation reactions of the significant carbohydrates, i. e., glucose, lactose, sucrose, dextrin, galactose, maltose, and mannite, were tested in Hiss serum water. From each culture diagnosed as positive on the Clauberg plates the pure strain was obtained and checked both for typical morphology by staining with acetic toluidin blue and by the usual fermentation reactions.

It was found that examination of the plates later than twenty-four to twenty-six hours was unreliable for after that time C. hoffmanni and a certain strain of staphylococcus would develop. These colonies appeared enough like those of C. diphtheriae to cause confusion in their identity. The size of the typical C. diphtheriae colony was found to vary in a marked degree, ranging on a average from ¾ mm. to 1 mm. and sometimes more in diameter. In practically all cases the C. diphtheriae was found to develop as a round smooth shiny and raised colony. By transmitted light the center of the young colony appeared as dark greyish-black extending over approximately one-third the whole area and becoming lighter towards the periphery. After twenty-four hours practically the entire colony became black. The diphtheroids, when they did appear, could, after some experience, be detected by the fact that the dark centers spread over a smaller area, were

TABLE I

SUMMARY OF RESULTS

	509 SWABS TESTED							
	ORIGINAL DIAGNOSIS				FINAL DIAGNOSIS AFTER FURTHER TESTS FOR IDENTIFICATION			
	LOEFFLER SLANTS		CLAUBERG PLATES		LOEFFLER SLANTS		CLAUBERG PLATES	
	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Positive	59	11.59	70	13.75	55	10.80	72	14.14
Questionable	2	0.39	8	1.57	0	0	0	0
Negative	448	88.41	431	85.68	454	89.20	437	85.86

blacker and the periphery did not have the characteristic whitish tinge of true diphtheria colonies. Some diphtheroid strains produced no dark centers at all. The streptococci, while developing clear cut dark centers, were not confusing because they were always flatter and more translucent than the C. diphtheria colonies and usually developed later. As noted by Clauberg and others there was one strain of staphylococcus which was not inhibited in its growth. However, it was found that this strain could be detected, after careful scrutiny, since the dark center extended further and the periphery was more transparent than that in the strains of C. diphtheriae colonies which grew typically large. In practically all the plates diagnosed as positive C. diphtheriae there were found to be no contaminating organisms. In over half the remaining plates in which C. diphtheriae was reported as negative no growth of any kind appeared.

RESULTS

The diagnosis made by culturing 509 swabs simultaneously on Loeffler slants and on Clauberg plates yielded the following comparative results, shown in Table I.

Examination of the tabulated summary in Table I reveals that prior to any confirmatory tests 11.59 per cent of all the cases tested were positive by Loeffler's

TABLE II

ANALYSIS OF FINAL RESULTS

	ACTUAL NUMBER	PER CENT	
		BASED ON TOTAL NO. OF CASES TESTED = 509	BASED ON TOTAL NO. OF POSITIVE CASES FOUND = 67
Total number of swabs diagnosed as positive. (Not including questionable cases)	67	13.16	100.0
Total number of swabs diagnosed as positive by both methods	53	10.41	79.10
Total number of swabs diagnosed as positive by Clauberg plates but not on Loeffler slants	13	2.55	19.40
Total number of swabs diagnosed as positive by Loeffler slants but not on Clauberg plates	1	0.19	1.49
Total number of swabs diagnosed as questionable positives on Clauberg plates only	8	1.57	11.94
Of which proved actually positive	4	0.78	5.97
Total number of swabs diagnosed as questionable positives on Loeffler slants only	2	0.30	1.22
Of which proved actually positive	1	0.18	1.49
Total number of proved errors by Loeffler's slants Five cases diagnosed negative but proved positive Nine cases diagnosed positive but proved negative	14	2.74	20.88
Total number of errors by Clauberg's method Three cases diagnosed positive but proved negative One case diagnosed negative but proved positive	4	0.78	5.97

slants and 13.75 per cent by Clauberg's plates. However, owing to questionable positive cases by both methods and to contradictory diagnoses in certain cases further tests for virulence and carbohydrate reactions were made upon the cultures in question. The correction of the mistakes and the confirmation of the questionable cases changed the final numbers of C. diphtheriae cases detected by Clauberg's and Loeffler's methods to 10.80 per cent and 14.14 per cent of all cases tested respectively.

Inspection of results shows that 67 swabs or 13.16 per cent of all the cases tested by one method or the other proved to be C. diphtheriae. However, only 53 of these positives were found to be so by both methods. Thirteen of the remaining 14 swabs were detected by examination on Clauberg's medium and were completely overlooked on Loeffler's slants. Only one strain of true C. diphtheriae which was reported on Loeffler's medium was not recognized on a Clauberg plate.

DISCUSSION

As may be seen from Table II the errors made from the macroscopic findings on Clauberg's medium amounted to 5.97 per cent of all the *positives* recognized by both methods or 0.78 per cent of all the swabs tested. These incorrect diagnoses consisted of: (1) Three swab cultures which were mistaken for C. diphtheriae but which proved to be negative by further tests, and (2) one swab culture considered negative but which was found to be positive later.

These four cultures all proved to be avirulent and by further confirmatory tests yielded the following results: (1) A streptococcus erroneously diagnosed as positive C. diphtheriae on a Clauberg plate. (2) A bipolar beaded rod with carbohydrate fermentations not typical of C. diphtheriae and diagnosed as positive C. diphtheriae on a Clauberg plate. (3) A solid rod with carbohydrate fermentations not typical of C. diphtheriae and diagnosed as positive C. diphtheriae on a Clauberg plate. (4) A bipolar rod with carbohydrate fermentation reactions typical of C. diphtheriae and with atypical growth on a Clauberg plate.

In each case the incorrect macroscopic diagnosis from Clauberg's medium was considered to be due to inexperience in recognizing C. diphtheriae on the plates before twenty-four hours' incubation had been completed.

On Loeffler slants the errors in diagnosis amounted to 20.88 per cent of all the positive forms found or 2.74 per cent of all the cases tested. These errors consisted of 14 cases, 5 of which were diagnosed as negative. On Clauberg's medium the growth was typical of C. diphtheriae and not only gave the correct carbohydrate fermentation reactions but were all virulent as well. The remaining 9 cases were diagnosed as positive C. diphtheriae from growth on Loeffler's slants but when cultured on Clauberg's medium yielded colonies giving the following results: (1) A solid rod which did not give the carbohydrate fermentation reactions typical for C. diphtheriae, growth atypical on Clauberg plate and found to be avirulent; (2) A solid rod with the fermentation reactions not typical of C. diphtheriae, atypical growth on a Clauberg plate and found to be avirulent; (3) A solid rod with carbohydrate fermentation reactions not typical for C. diphtheriae, atypical colony on a Clauberg plate and found to be avirulent; (4) A bipolar rod, giving no carbohydrate fermentation reactions typical of C. diphtheriae, atypical colony on a Clauberg plate and avirulent; (5) (6) Two cases in which but scant growth could be obtained after forty-eight hours and which proved to be streptococci only; (7) (8)

(9) All failed to grow on Clauberg plates both cultured directly from the original swab and also from the original growth on Loeffler's slants.

It is true that these 9 cases which gave negative results on Clauberg's medium are proof of error by the Loeffler method in a negative way only, for the original Loeffler swab was not tested for virulence. Experience has shown that true C. diphtheriae will develop on Clauberg plates regardless of how heavily contaminated the material and for this reason it has been felt that these results should not be disregarded.

The 8 swabs which resulted in questionable growth on Clauberg's medium were diagnosed as typically negative by the staining of growth on Loeffler's slants. Further examination of isolated colonies from the plates proved that 4 of these were virulent C. diphtheriae strains yielding typical fermentation reactions on the carbohydrates. Each strain appeared to have typical bipolar rods after growth on Loeffler medium. The 4 remaining cultures all proved to be avirulent, comprising (1) a barred club form with atypical sugar reactions; (2) a strain resembling C. hoffmanni morphologically and by the sugar reactions; (3) a solid resembling atypical sugar reactions; (4) a strain which was beaded and bipolar and which gave the typical reactions of C. diphtheriae on the carbohydrate tests.

On Loeffler's slants only two cultures were found to be questionable C. diphtheriae and to require further tests to confirm the diagnosis. One of them was typically positive on Clauberg's medium and proved to be both virulent and to give the typical fermentation reaction of C. diphtheriae on the carbohydrates. The other culture failed to grow on Clauberg's medium.

CONCLUSIONS

1. In spite of the fact that more questionable cases occurred on Clauberg's medium than on Loeffler's medium the total number of actual C. diphtheriae detected solely by eye was significantly greater by the former method than by the latter. It may be concluded that substitution of the macroscopic method of colony identification for the usual microscopic examination of smears, saves time without impairing the accuracy of the diagnosis.

2. We consider that the success of macroscopic diagnosis by Clauberg plates for the routine diagnosis of diphtheria depends to a great extent upon one factor, namely, the experience of the worker. In our opinion the plates are useless to one who has never worked with them before. It is believed that sufficient experience may be gained after two or three weeks to enable one to diagnose typical plates with confidence and greater accuracy than by the usual stains of smears from Loeffler slants.

3. The only disadvantages connected with the mediums lie (1) in the difficulty of keeping a sufficient number of plates on hand, and (2) in the expense of keeping a supply of fresh sheep blood available. The latter would necessitate either the purchasing of fresh sheep blood at the time it was required or the maintenance of a sheep for bleeding purposes. The comparative cost of equal volumes of Clauberg's and Loeffler's mediums has been found to be approximately the same. It is suggested that small Petri dishes be used since large plates are unnecessarily wasteful of the medium unless two swabs may be streaked on one plate.

4. One of the chief advantages of the medium aside from the differential antiseptic action of the tellurite lies in the fact that isolation by streaking these plates

not only permits diagnosis by means of the usual staining procedure but also makes possible the study from pure cultures. The time involved is no longer than the usual routine procedure.

REFERENCES

1. Loeffler, F.: Bedentung der Mikroorganismen für die Entstehung der Diphtherie beim Menschen, bie der taube und beim Kable, Mitt. Gesundh. Amt. **2**: 421, 1884.
2. Conradi and Troch: Ein Verfahren Zum Nachweis der Diphtherie-bazillen, München. med. Wchnschr. **59**: 1652, 1912.
3. Smith, J. F.: Isolation of B. diphtheriae by Means of a Simple Medium Containing Potassium Tellurate, J. Path. & Bact. **19**: 122, 1914.
4. Pergola, M.: Substrati al tellurito e Colorazione ridotta nella re cercal dimostrazione del bacillo difterico, Boll. d. Inst. Sieroterap. Milanese **7**: 585, 1928.
   Recherche et demonstration du bacilli diphterique moyenment le serum-oeuf-tellurite (S. U. T.) et la coloration reduite, Boll. della Sez. Italiana **1**: 217, 1929.
5. Douglas, S. R.: A New Medium for the Isolation of B. Diphtheriae, Brit. J. Exper. Path. **3**: 263, 1922.
6. Allison, V. D., and Ayling, T. H.: An Improved Medium for the Isolation of Cornybacterium Diphtheriae; Trypsinized Serum Tellurite Copper Sulphate Agar, J. Path. & Bact. **32**: 299, 1929.
7. Clauberg, K. W.: Ueber einen elektivnahrboden für Diphtheriebazillen, der eine makroskopische plattendiagnostik ermöglicht, Zentralbl. f. bakt. **114**: 539, 1929.
8. Clauberg, K. W.: Weiter Mitterlung zur makroskopischen Diphtheriebazillen diagnostik, Zentralbl. f. bakt. **120**: 324, 1931.
9. Anderson, T. S., Happold, F. C., McLeod, S. W., and Thomson, J. G.: On the Existence of Two Forms of Diphtheria Bacillus—B. Diphtheriae Gravis and B. Diphtheriae Mitis—and New Medium for Their Differentiation and for Bacteriological Diagnosis of Diphtheria, J. Path. & Bact. **34**: 667, 1931.
10. Pesch, K. L., and Krämer, E., Der, K. T. C.: Blutagar ein neuer anreicherungsnahrboden für Diphtheriebakterien, Klin. Wchnschr. **9**: 119, 1930.
11. Manzini, C.: Les millieux au tellurite de potassium et sans tellurite, comme moyen d'isolement et d'identification macroscopique des cultures du bacille diphterique, Boll. d. zez. Ital. Soc. Intern. di Microbiolog. **2**: 451, 1930.
12. Tempe, G.: Culture de bacille diphterique sur milieu au tellurite de potassium, Compt. rend. Soc. d. Biol. **104**: 1341, 1930.
13. Seistrop, J. G.: Is op het oogenblik verbetering van de bacteriologische diphtheriediagnostick mogelyk, Ned. Tijdschr. v. Geneesk. **74**: 4104, 1930.
14. Tallo, F.: Sulla diagnosi batteriologica difterite e la sorte del B. difterico nei portatori convalescenti e sani, Gior. di Batteriol. e. **6**: 285, 1931.
15. Force, J. N., and Beattie, M. I.: Intracutaneous Test for Virulence of Diphtheria Bacilli in Field Cultures, Am. J. Hyg. **2**: 490, 1922.

COMPARISON OF THE KOLMER MODIFICATION OF THE WASSERMANN AND THE KLINE YOUNG PRECIPITATION TESTS*

LESLIE C. TUCKER, PH.G., JACKSON, MISS.

A COMPARISON of the Kolmer Wassermann, using two antigens, the Kolmer and the Acetone Insoluble, with the Kline Young Precipitation Test, on 3390 routine bloods gave the following results:

3041—complete agreements or approximately	89.4%
52—4 plus Kolmer and negative Kline or approximately	1.6%
96—negative Kolmer and 4 plus Kline or approximately	2.9%
141—negative Kolmer and 2 plus Kline or approximately	4.2%
23—2 plus Kolmer and negative Kline or approximately	0.8%
37—anticomplimentary or approximately	1.1%
3390—Total number of cases	100.0%

*From the Laboratories of the Mississippi State Hospital.
Received for publication, April 20, 1932.

In the cases showing 4 plus Kolmer and negative Kline, using the Kolmer Modification of the Wassermann, the spinal fluids were as follows:

17—positive or approximately	41.5%
24—negative or approximately	58.5%
11—No punctures	
52—Cases.	

In those cases showing negative Kolmer and 4-plus Kline, using the Kolmer Modification of the Wassermann, the spinal fluids were as follows:

29—positive or approximately	36.3%
51—negative or approximately	63.7%
16—No punctures	
96—Cases.	

CONCLUSIONS

1. The Kline reaction may appear to be slightly more sensitive in blood serology.

2. In two small series in which there was a total disagreement between the Kolmer and the Kline Young reactions on the blood, there was approximately the same proportion of positive spinal fluids, using the Kolmer reaction as a basis.

3. Both tests should be run as a routine procedure in the laboratory in order that no positive may escape.

A SIMPLE CONSTANT INJECTION APPARATUS*

SAMUEL SOSKIN, M.D., PH.D., CHICAGO, ILL.
WITH THE TECHNICAL ASSISTANCE OF HANS WILSON

FOR some time we have been using a simple constant injection apparatus of our own design, activated by a windshield wiper of the pressure type. This apparatus is simple and economical in construction, accurate and positive in action, and has the further advantage that it automatically registers the amount of solution injected.

The apparatus consists of a ¾ inch square bronze block, 4 inches in length, through the long axis of which a ⅜ inch hole has been bored. Through this hole a sliding-fit rod operates with a to and fro motion. The rod is fastened by a cross-member at each end to a guide bar, which slides through holes in the supporting posts of the square block. At each stroke of the windshield wiper a small crank attached to the windshield wiper shaft pushes a post on the guide bar. This causes the spring pawl at one end of the sliding rod to engage in the rachet wheel. As the windshield wiper reverses its direction the sliding rod is returned to the resting position by a compression spring at its opposite end. An adjustable collar at the pawl end of the sliding rod determines its resting position and allows for the adjustment of the stroke. The rachet wheel is supported by two pillar blocks and its center is finely threaded to receive a long threaded bar. A channel and pin prevent the threaded bar from rotating while the apparatus is in motion, so that the move-

*From the Metabolism Laboratory, Department of Physiology, Michael Reese Hospital, Chicago.
Received for publication, April 1, 1932.

ment of the rachet wheel causes the threaded bar to push the piston of the syringe. The pin may be released to allow the threaded bar to be quickly returned to its original position when the syringe is to be refilled. The syringe is firmly held in a divided wooden block held together by thumb screws. At each stroke of the windshield wiper a crank attached to its shaft operates a counting device.

Although this apparatus is particularly suitable for slow rates of injection, the injection speed can be almost infinitely varied by adjusting (a) the size of the syringe used, (b) the strength of the solution used, (c) the length of the stroke, (d) the speed of the windshield wiper (regulated by means of a needle valve). When great accuracy is required the syringe to be used may easily be calibrated by causing it to deliver into measuring flasks or weighing bottles. The amount of solu-

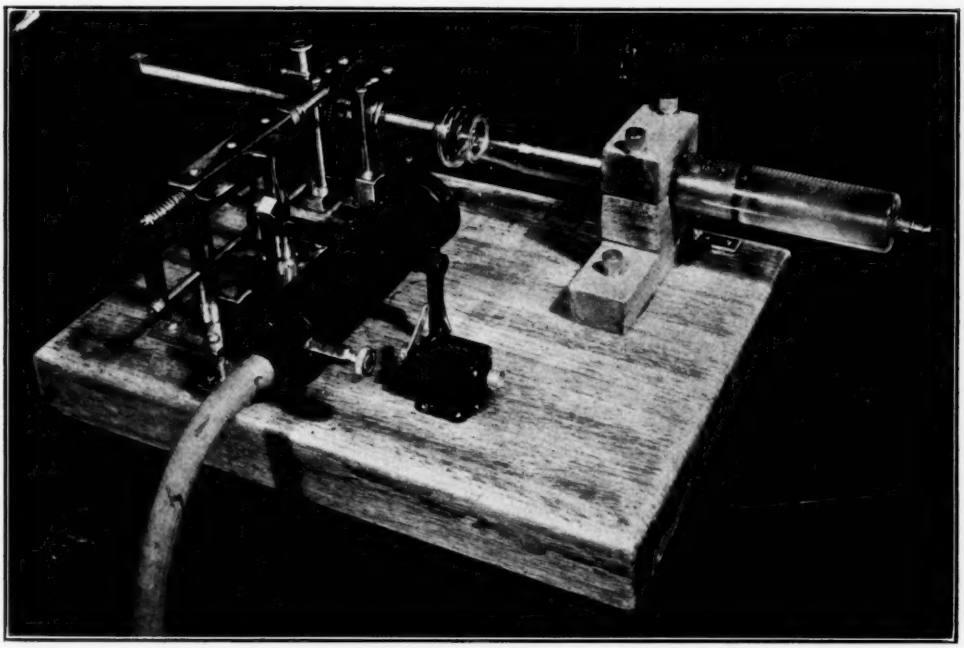


Fig. 1

tion delivered at each stroke of the windshield wiper (recorded on the counting device) can then be calculated. We have used as our source of air pressure a compression pump and storage tank system, which delivers air at 30 to 35 pounds pressure, depending upon the number of outlets simultaneously in use. With this source of pressure our injection apparatus has repeatedly run eight continuous hours without regulation, with a total error of about 2 per cent in the amount of solution injected in that period. By periodic timing and adjustment of the windshield wiper this error can be reduced to almost zero.

This apparatus has a distinct advantage for the injection of sterile solutions in that only the syringe and the tubing and needle attached to it need be sterilized. It can be made portable and hence used outside the laboratory by making use of tank of compressed gas to run the windshield wiper.

We wish to acknowledge our indebtedness to Fred Overthun for assisting in the construction of this apparatus.

LABORATORY AIDS*

RAYMOND H. GOODALE, M.D., WORCESTER, MASS.

EVERY laboratory develops certain conveniences which meet its particular needs. Many of these devices can be adopted by other laboratories with very little expense. The articles which are described here were developed in this laboratory as the needs arose. They have worked out very satisfactorily, and so I am passing them along to others.

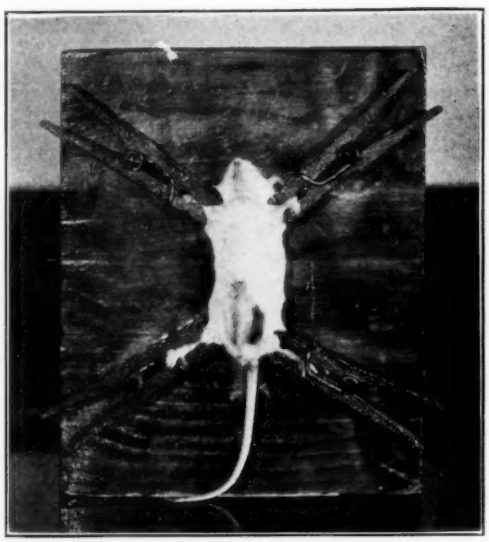


Fig. 1.—Mouse board.

1. MOUSE BOARD.

A simple and inexpensive mouse board was made by nailing four spring-type clothespins to a piece of board measuring 8 inches by 8 inches. The position of the legs of the average-sized mouse used is outlined on the board. The clothespins are then nailed so that the claws will be in the right position to seize the legs (Fig. 1). The clothespins are nailed with a single nail through the spiral spring in the center. This allows the clothespins to be adjusted to the size of the animal. A larger type of this board can also be used for guinea pigs.

2. STAIN ILLUMINATOR

We have wasted considerable time in locating pieces of sectioned tissues in the dark stains such as hematoxylin and Geshichter's "metachrome 136." To avoid this loss of time we set the staining dish on the illuminating table seen in Fig. 2.

*From the Pathological Laboratory, City Hospital.
Received for publication, April 25, 1932.

This is simply a square piece of window glass in a frame 6 inches by 6 inches and supported by four legs four inches high. The light is furnished by a 25 watt bulb fastened under the glass. This reduces the time consumed in staining and assures one of finding all of the pieces of tissue.

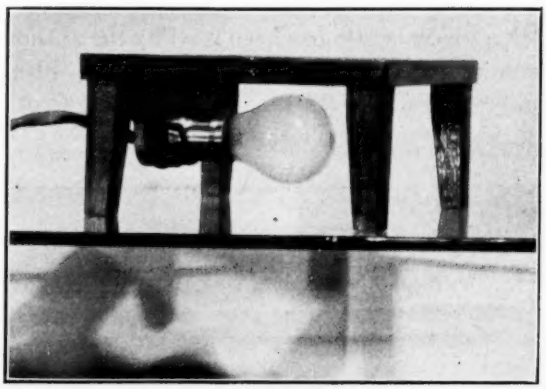


Fig. 2.—Stain illuminator.

Fig. 3.—Measuring knife.

3. MEASURING KNIFE

In describing surgical and autopsy tissues a ruler is necessary. In order to measure and cut tissues quickly the ruler and knife combination shown in Fig. 3 was devised. It was made by cutting off the centimeter side of a 15 centimeter celluloid ruler and attaching it to the flat back of a knife with china cement.

A DOG-MASK FOR RESPIRATION EXPERIMENTS*

H. F. Pierce, Ph.D., Baltimore, Md.

THE type of mask here described has been used by the author for various respiration experiments during the past ten years, and was designed to provide a simple means of making masks for all sizes of dogs, goats, and other animals having long snouts. The mask is made from a tin can, a short piece of one-half inch brass

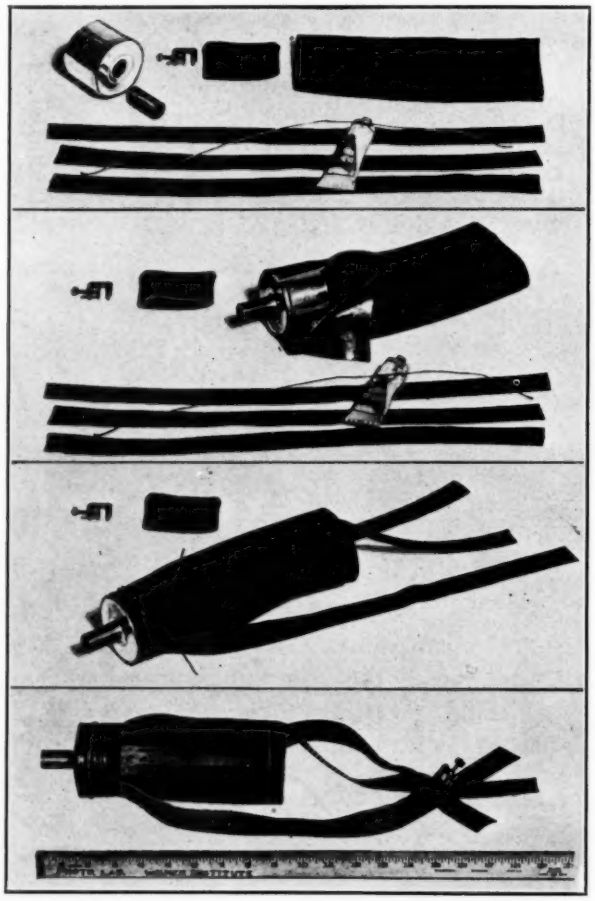


Fig. 1

Fig. 2

Fig. 3

Fig. 4

tubing, and a section of automobile tire inner tube (Fig. 1). Food tins may be had in a great variety of sizes, and old inner tubes from 2 inches to 7 inches in diameter may be obtained at any tire repair shop. The inner tube should be chosen to fit the animal's nose tightly at a point just below the eyes and behind the corners of the mouth. The tin should be large enough not to press upon the sensitive parts of the animal's nose.

*From the Wilmer Institute of the Johns Hopkins University and Hospital.
Received for publication, May 4, 1932.

The tin is cut down to a length of 2 inches to 3 inches, a hole made in the end, and a short piece of brass tubing soldered into the hole, care being taken that no sharp projections remain inside the tin. If the inside of the inner tube is rough it should be turned inside out, so that the inner surface of the mask will be smooth. The inner tube is split lengthwise for a distance of 4 inches and the flaps sandpapered, treated with rubber cement, and wrapped tightly around the tin (Fig. 2). A small opening between the tin and the rubber remains where the lap occurs, and should be filled with cement to make the joint air-tight.

Three strips 1 inch wide and 18 inches long are cut from an inner tube and cemented, at one end, to the mask, 120° apart (Fig. 3). Two turns of No. 16 B and S gauge copper wire are wound tightly over these joints, and, last, a piece of inner tubing the size of the tin is slipped over the wire and cemented in place.

If the animal is not of the short-haired variety, it is advisable to clip the hair about the muzzle before applying the mask. The hair should then be rubbed full of heavy petrolatum and the mask slipped over the nose. The long elastic straps fix the mask in position. One passes between the animal's eyes and over the top of the head, and the other two along the angles of the jaw and under the ears to the back of the head, where the three are fastened together by means of a Hoffman clamp (Fig. 4). After the mask is fitted and the straps drawn tight, any possible openings below the eyes or under the jaw should be filled with petrolatum. This has been found to maintain a tight joint up to a pressure of 1 inch of water.

A NEW METHOD OF MOUNTING EYES AND OTHER SMALL MUSEUM SPECIMENS*

ANDREW A. EGGSTON, M.D., NEW YORK, N. Y.

THE following method for the mounting of museum specimen of eyes and small specimens have been used at the Manhattan Eye, Ear and Throat Hospital for a number of years and has proved of so much satisfaction as to justify its publication.

I. Fix the enucleated eye or specimen in Klotz solution after having made a small slit through the optic nerve in the case of an eye with a razor blade. Leave the specimen in this fixing solution for six to seven days.

II. Halve the eye with a razor blade starting from the slit originally made in the optic nerve, sectioning the eye downward by a short sawing motion, until the blade arrives at the level of the lens. Then seize the razor blade at each end firmly with thumb and index fingers of both hands and press down firmly until lens is severed and the globe is completely halved.

III. Place the half of eye to be mounted in 70 per cent alcohol for thirty minutes; 95 per cent for thirty minutes and absolute alcohol for a few minutes. Drain and fix on perforated celluloid plaque bearing name, number and diagnosis written with India ink. Fasten eye to plaque by means of a coat of celloidin around edge of hole. Let dry for several minutes.

*Received for publication, May 10, 1932.

IV. Immerse the celluloid sheet with the specimen attached in special jar filled with Kaiserling III and bend plaque so as to have upper edge of it press against neck of jar. Close with tight fitting cover and clamp. A metal clamp is attached to the jar which will support one slide of a mounted section of the eye, thus completing the record of the specimen.

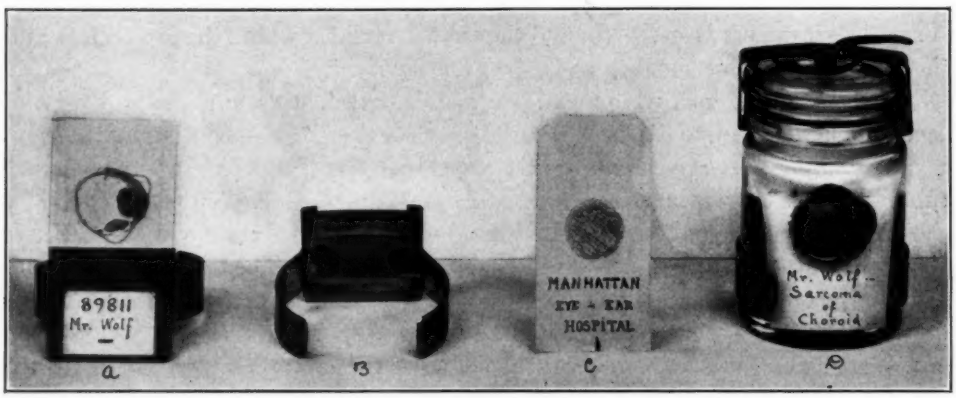


Fig. 1.—*A*, Slide clip with slide in place. *B*, Slide clip. *C*, Celluloid sheet with hole for specimens and title. *D*, Eye mount complete.

The advantages of this method consist of preservation of natural color. Immobile fixation of specimen to celluloid sheet in the jar so that the jar may be handled in a very rough manner even set upside down and the specimen remains undisturbed. No deterioration of medium and air bubble formation as occurs in the gelatin mount so long used in museum preparation of eyes.

The materials necessary for the mounting may be obtained from the Apex Scientific Equipment Co., 92 Monroe Street, New York City.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TISSUE: A Time-Saving Procedure for Embedding In Paraffin, Baumgartner, W. J., and Welch, B. M. Stain Techn. **7**: 129, 1932.

Short sections of rubber tubing are set on a glass slide, a piece of plain window glass an inch or more square, or the tube may be set into a watch crystal. The glass is rubbed with glycerin and the tubing cooled before using. The tubing is set upon the glass and warm paraffin run into the rubber container. The paraffin in contact with the glass and rubber cools slowly and hardens. Meanwhile, the specimen is placed and oriented in the center. Blowing a little air over the top will form a congealed film. The entire preparation is lowered carefully but quickly into cold water. As the paraffin cools it shrinks but the rubber tubing yields on all sides. Thus the shrinkage is not all from the top and so does not cause the "center dip" commonly resulting in solid containers. When fully hardened, the block is removed from the slide and the rubber separated from the round, well-formed block of paraffin, which may then be labelled. The tubing and glass are again ready for use.

The size of the rubber tubing and the length of the sections to be used will vary with the size of the piece to be embedded. The thickness of the rubber may vary; the only essential being that the tube shall maintain its shape. The authors recommend tubing of 10 and 20 mm. in diameter, and sections cut 7 and 14 mm. in length. A good way to cut the rubber tubing so that the sections will have smooth, straight edges is to place it on a round rod of wood, which is then put into a lathe. The tubing is now cut readily into sections with a sharp knife while the wood is turning.

BROMIDE IN BLOOD: Determination of, Finner, L. L. Clifton Springs Med. Bull. **18**: 114, 1932.

Whole blood, plasma, or serum may be used, and only 2 c.c. are required. The proteins are removed by the procedure of Folin and Wu. The filtrate is treated with a knife-point full of powdered animal charcoal, shaken for a minute, and refiltered. To 10 c.c. of filtrate is added 0.5 c.c. of 1 per cent gold chloride solution, and the color is compared after one minute and within 10 minutes with that obtained by treating 10 c.c. of bromide solution of appropriate strength with the same amount of gold chloride solution. Without the charcoal treatment, occasional filtrates develop a turbidity on adding the gold chloride. A stock standard solution containing 0.3 mg. bromine per 100 c.c. is convenient. From 1 c.c. to 10 c.c. of the stock solution are introduced into a series of test tubes, and the volume in each tube is made up to 10 c.c. Such a series covers blood bromine ranges from 30 mg. to 300 mg. per 100 c.c. The standard bromide solution should be titrated against silver nitrate.

At concentrations as high as 300 mg. bromine per 100 c.c. blood, the error does not exceed −10.0 per cent, and in the concentration usually encountered clinically, added bromide can be recovered quantitatively. When the concentration is less than 30 mg. bromine per 100 c.c. blood, the color becomes too faint for accurate comparison. Except for iodides, which also give a brown color with gold chloride, the reaction is apparently specific for bromides.

B. **TUBERCULOSIS, Tissue Substrate Microculture for Tubercle Bacilli,** Corper, H. J. J. A. M. A. **99**: 1315, 1932.

The method consists of placing from 0.5 to 1 c.c. of well ground-up or finely divided suspected specimen (sputum, tissue, etc.) in a sterile bacteriologic test tube, 6 by ⅝ inch, or 6 by ¾ inch, stoppered with a cork, after sterilization by dipping the end to be inserted into the tube into hot sterile paraffin. To the suspected specimen is added a few drops (0.5 c.c.) of sterile citrated (1/10 volume of sterile 3 per cent neutral trisodium citrate) human blood (other bloods

if available will also do) or 0.5 c.c. of fresh egg yolk. Blood appears more suited than egg yolk, primarily because of the more scattered skein colonies growing in the blood, although egg yolk has proved satisfactory as a nutrient in control tests. To the well mixed specimen and nutrient is added about from 1½ to 2 volumes of 6 per cent sulphuric acid which is intimately mixed by shaking, and the acid preparation is then placed in the incubator at 37° C. for about forty-five minutes (a range of from one-half to one hour is admissible), being vigorously shaken occasionally during incubation. After removal from the incubator, the acid is neutralized by the cautious addition of a volume of sterile 1.3 per cent pure sodium bicarbonate solution containing 3 per cent pure glycerin previously determined adequate to neutralize the amount of 6 per cent sulphuric acid used. Bromthymol blue, 0.04 per cent, was used as indicator and a deep green blue (P_H 6.8) or light blue (P_H 7.4) color was found to be satisfactory, following Clark's description and technic of making the tests. Neutralization can also be performed with sodium hydroxide, although the danger of passing the neutral zone is greater and it does not act as efficiently as sodium bicarbonate as a buffer during incubation. After neutralization, the tissue substrate is allowed to settle overnight in the refrigerator or the mixture can be centrifugated at low speed, after which the supernatant liquid is decanted, leaving a small portion behind (from 0.5 to 1 c.c. above the sediment) to serve in readily shaking and making smears when required; a 2 to 3 c.c. total volume of tissue substrate and liquid suffices when the original specimen including nutrient was from 1 to 1.5 c.c. in amount. After shaking to break up the sediment, the tube is carefully stoppered and is placed in a dark incubator at 37° C. After shaking the tubes well on removal from the incubator, smears are made at weekly or biweekly intervals on clean slides covered with a thin film of Mayer's albumin fixative, and after carefully fixing by heat, they are stained with steaming carbolfuchsin according to the Ziehl-Neelsen technic for acid-fast bacilli, precautions being taken not to wash off the smear during staining and decolorizing.

PLASMA-PROTEIN, Simple Test for Below The Edema-Producing Level, Page, I. H., and Van Slyke, D. D. J. A. M. A. **99**: 1344, 1932.

Reagent.—Xylene, 1 volume; monochlor benzene 2.06 volumes. One drop of a 3.36 per cent sodium chloride solution (Sp. grav. 1.0235) should neither rise nor fall but float in the mixture.

The Test.—The freshly drawn blood is mixed with heparin, 1 mg. per cubic centimeter of blood, or with oxalate, not more than 2 or 3 mg. per centimeter, and is centrifugated. The clear plasma is dropped from a pipette held about 2 cm. above the level of the fluorobenzene or xylene-chlorobenzene mixture. If the plasma specific gravity is below that of the fluid, the drop will rise rapidly to the surface, whereas if the drop has a higher specific gravity, it will fall rapidly to the bottom. Plasma just at the critical edema level will bob around if the tube is gently shaken, seemingly unable to decide whether to rise or fall. After a short time the drop flattens on the side or bottom of the tube and remains there as a record of its specific gravity.

PNEUMONIA, A Study of Lobar Pneumonia in Massachusetts: Methods and Results of Pneumococcus Type Determination: 1931-1932, Heffron, R., and Varley, F. M. Am. J. Pub. Health **22**: 1230, 1932.

1. Pneumococcus typing is being requested by increasing numbers of physicians in Massachusetts.

2. During the past winter all 32 (Cooper) types of pneumococci excepting Types XXV and XXXII were found.

3. There was no especial geographical distribution evident of any of the types found.

4. Multiple cases of lobar pneumonia occurring in the same family at approximately the same time were very uncommon.

5. The Krumwiede, Sabin and tube agglutination methods of typing were and still are largely used and were found to be over 99 per cent accurate when definitely positive.

6. The Sabin method of typing has given a greater number of accurate positive typings in a shorter period of time than any other method studied, and is a method readily learned by technicians inexperienced in this work.

7. Of 789 specimens typed at the State Bacteriological Laboratory during the 11

months, September 1, 1931, to July 31, 1932, the type of pneumococcus found was checked in 94.6 per cent of the cases by a second method of typing or from a culture of the organism.

8. The various sub-types (Types IV to XXXII) were found more frequently during the 6 winter and spring months, December to May, than during the warmer months.

9. In view of the results to date, and until conclusive proof to the contrary is obtained, it is felt that every type determination done by the Krumwiede, Sabin, or tube agglutination method should be checked by some other method of typing. Typing the organism obtained from cultures of the mouse heart's blood affords the most valuable means of checking.

ERYTHROCYTE VOLUME, Relation of Adrenal Glands to, Bon Haam, E., and Thatcher, H. S. Endocrinology **16**: 666, 1932.

From an experimental study the authors conclude that:

1. The intravenous administration of adrenaline, asphyxia and bleeding produced a short lasting increase of the erythrocytes in the peripheral blood of 20 dogs. The autopsy revealed in all cases a marked contraction of the spleen during the time the hematocrit values in the peripheral blood were increased.

2. Adrenalectomy prevented or hindered the reflex which leads to the splenic contraction and to the acute erythrocytosis in the peripheral blood after asphyxia and bleeding.

3. It is concluded that the adrenal glands or their secretion, adrenaline, play an important part in the reflex mechanism which regulates the distribution of erythrocytes in the body.

SYPHILIS, Serology of From the Standpoint of the Public Health Laboratory, Kolmer, J. A. Am. J. Pub. Health **22**: 1253, 1932.

The paper is summarized below:

1. A choice of method or methods for the serum diagnosis of syphilis is a problem of special interest to public health laboratories.

2. Of particular importance to all laboratories and especially those engaged in public health work without the advantage of skilful clinical checking of reactions is the question of the specificity of weakly and unexpectedly positive reactions.

3. What is particularly required is the use of a test or tests possessing only the maximum of sensitiveness consistent with specificity in order to earn the confidence of the medical profession in the significance of weakly positive reactions.

4. By employing a test or tests possessing the maximum of sensitiveness consistent with specificity, public health laboratories can do a great deal in minimizing the regrettable error of insufficient treatment of syphilis.

5. Precipitation tests for syphilis are more economical than the Wassermann test since a hemolytic system is not required but they demand an equal degree of skill and one more subject to error and interpretation of weakly positive and doubtful reactions.

6. Not infrequently the various precipitation tests for syphilis have been compared with complement fixation tests lacking in acceptable sensitiveness and specificity and consequently resulting in erroneous conclusions.

7. Despite strenuous efforts none of the precipitation tests can be said to have displaced complement fixation tests possessing an acceptable degree of sensitiveness and specificity when judged impartially and scientifically with adequate clinical control. The use of two or more precipitation procedures has not proved as satisfactory as complement fixation and precipitation tests of acceptable sensitiveness and specificity for the routine testing of sera.

8. It is impossible at present to make a choice of a single precipitation test from among the large number available.

9. The new Meinicke or "clarification" test has been found more sensitive than the Kahn test, of equal specificity and easier to read and interpret the reactions.

10. In a comparative study of the Kline microscopic and Kahn precipitation tests and the Kolmer modification of the Wassermann test, the reactions agreed in 86.7 per cent of over 1,000 sera and disagreed in 13.3 per cent.

11. In general terms the Kline microscope test was more sensitive than the Kahn and Kolmer tests but likewise gave a higher percentage of nonspecific or falsely positive reactions than the Kahn test while the Kolmer modification of the Wassermann test gave no falsely positive reactions.

12. At the present time the serum diagnosis of syphilis is best served by conducting a carefully chosen complement fixation and precipitation test on each serum.

LIVER, Functional Capacity of: Comparative Merits of the Five Most Popular Tests, Robertson, W. E., Swalm, W. A., and Konzelmann, F. W. J. A. M. A. 99: 2071, 1932.

From a fairly extensive study the authors conclude that for routine procedure the icterus index is one of the most valuable tests for liver function. The chief value of the van den Bergh lies in its selectivity for bilirubin, while the bromsulphalein test, especially when (in cases without jaundice) the 5 mg. dose is used, most frequently agrees with the clinical picture.

They were not impressed with either the galactose tolerance or urobilinogen tests.

BLOOD: Study of "Iron Volume Index" and Its Significance in the Treatment of Anemia, Reich, C., and Tiedemann, V. G. Am. J. M. Sc. 184: 637, 1932.

The following method is given for the determination of the new index proposed:

Twenty cubic centimeters of blood are withdrawn from the arm vein and 10 c.c. are run into a 15 c.c. graduated centrifuge tube containing 2 c.c. of a 1.6 per cent sodium oxalate solution. This is mixed by inverting, and centrifuged at 2500 r.p.m. for one hour. The total cell volume per cent is then read off and calculated for 100 c.c.

The other 10 c.c. are shaken up in a test tube previously prepared by evaporating 2 c.c. of 1.6 per cent iron-free sodium oxalate solution to dryness. The sodium oxalate used was obtained from the Bureau of Standards and shown by controls to contain no iron. This blood is used for hemoglobin, red blood cell and iron determinations. The hemoglobin was done by both the Sahli and Newcomer methods in each case, and 15.6 gm. of hemoglobin were taken as 100 per cent. Red blood cell counts were done with two standardized pipettes, a drop from each being counted for every determination, and the two averaged. Results were not accepted if the error was more than 2 per cent.

Iron is determined as follows:

Standard Iron Solution: Standard A: Dissolve 0.702 gm. ferrous ammonium sulphate in about 50 c.c. of water. Add 20 c.c. of 10 per cent iron-free sulphuric acid. Warm and then add N/10 potassium permanganate until a faint pink color persists. Dilute to 1 liter; 1 c.c. will contain 0.1 mg. of iron. *Standard B:* Standard A diluted with an equal volume of water; 1 c.c. will contain 0.05 mg. of iron.

Sulphocyanic Acid: One hundred grams of ammonium thiocyanate are dissolved in 100 c.c. of 65 per cent by weight sulphuric acid. Measure volume, transfer to separatory funnel and shake out immediately with three-fourths volume of amyl alcohol. Discard the aqueous solution and again shake out twice with an equal volume of water. Combine the water extracts, which together contain about 7 per cent of sulphocyanic acid. Saturate with mercuric sulphocyanate so that some remains on the bottom. Allow to stand over night in a dark bottle.

Persulphate Solution: A saturate solution of C.P. iron-free sodium persulphate in distilled water.

Extraction Mixtures: (1) Five parts of amyl alcohol and 2 parts of ether; (2) equal parts of ethylene glycol monobutyl ether and ethyl ether.

The ether does not have to be of special purity, as the presence of mercuric sulphocyanate prevents the oxidation of the sulphocyanic acid by the peroxids in the ether.

Iron Determinations. One cubic centimeter of blood is diluted with 4 c.c. of water and 1 c.c. of the mixture is digested according to the method of Wong. After being allowed to cool it is diluted to approximately 10 c.c. and a drop of persulphate is added, followed by exactly 25 c.c. of the extraction medium and 5 c.c. of the sulphocyanic acid reagent. The tube is then tightly fitted with a rubber stopper, the mixture is well shaken and after a few minutes' standing separates into two distinct layers. If this separation does not take place

rapidly it can generally be accelerated by the addition of a few cubic centimeters of water. One cubic centimeter each of Standards A and B are then digested and treated in the same manner. The A Standard is suitable for figures within the normal range (35 to 50) and the B Standard is used where the iron values are lower than the normal. Since the A Standard contains 0.1 mg. of iron and the B Standard 0.05 mg. of iron, and 0.2 c.c. of blood are used, the formula for figuring the iron is as follows:

FORMULA FOR FIGURING IRON

$$S \times \frac{Rs}{Rx} \times 500 = \text{mg. of iron.}$$

S = strength of standard.
Rs = reading of standard.
Rx = reading of unknown.
Calculations:

$$\text{Volume Index} = \frac{\frac{Cv}{46}}{\frac{RV}{5.0}} \quad \begin{matrix} \text{Total cell volume, per cent} \\ \text{Normal cell volume, per cent} \\ \text{R.B.C. in millions} \\ \text{Normal R.B.C. in millions} \end{matrix} \quad \begin{matrix} \text{Normal Values} \\ \frac{46}{46} \\ \frac{46}{5} \\ \frac{5}{5} \end{matrix} = 1$$

$$\text{Saturation Index} \frac{\frac{Hb\%}{100}}{\frac{Cv}{46}} \quad \frac{\frac{100}{100}}{\frac{46}{46}} = 1$$

$$\text{Corpuscular iron index} = \frac{\frac{I}{45}}{\frac{Cv}{46}} \quad \begin{matrix} \text{Iron in mg. per 100 c.c.} \\ \text{Average iron in mg. per 100 c.c.} \end{matrix} \quad \frac{\frac{45}{45}}{\frac{46}{46}} = 1$$

$$\text{Average volume of erythrocyte (Ev)} = \frac{Cv \times 10^{12}}{Rm \times 10^{11}} \text{ cubic micron} \quad \frac{46^{12}}{5^{11}} = 92$$

Average Hb per erythrocyte (Hbe)* = Hb (gm.) × Ev × 10^{-14} = (15.6 × 92) = 143
Average iron per erythrocyte (Ie)* = I × Ev × 10^{-14} = $(45 \times 92)^{-3}$ = 4.4

The normal figure for this index is between 0.9 and 1.0 and when this index is 1.0 the red cells are regarded as saturated with iron.

An iron volume index of 1.0 is obtained in most normal people and in cases of pernicious anemia, leucemia and in secondary anemias associated with carcinoma and acute and chronic infections with fever. Iron therapy seems to be of no value in these cases, due to the fact that the red cells are already saturated with iron.

An iron volume index of less than normal is most commonly found in cases of secondary anemia caused by malnutrition or loss of blood. Iron is of decided therapeutic value in the treatment of these patients.

HEMOLYTIC SERA, Preparation of, Strafseth, H. J. Science 76: 444, 1932.

The usual directions for the preparation of hemolytic and precipitating sera call for the use of washed red blood cells and serum respectively as antigens.

The fact is, however, that an immune serum, prepared by using clear serum as antigen will function perfectly as a hemolytic serum and that a hemolytic serum prepared by using washed red blood cells as antigen will do very well as a precipitating serum.

*In the table these determinations, namely, average Hb and average iron, are expressed as × 10^{-11} by pointing off three more places.

It is necessary to heat sheep serum for one-half hour at 56° C. and to remove fresh serum from the red blood cells by repeated washing in saline solution.

Sterile serum or plasma can be kept for months or years, thus offering a saving in time and effort, especially to those who may wish to work with hemolysins and precipitins without having ready access to sheep or goats.

Giving rabbits 2, 3, 4 and 4 c.c. of sheep serum intravenously, allowing three to four day intervals between injections and eight to ten days between the last injection and the bleeding, has produced sera which, when used as precipitins, showed titers of better than 1:12800. The same sera used as hemolysins dissolved 0.5 c.c. of a 2 per cent suspension of sheep erythrocytes in quantities of 0.01 c.c. of a 1 per cent dilution and of 0.05 c.c. of a 0.5 per cent dilution in the presence of 2 units of complement in thirty minutes at 37° C.

Rather strong hemolytic and precipitating sera have also been obtained by using as antigen the clear saline solution in which blood cells had been washed, the solution from the fourth washing being about as effective antigenically as that from the first one.

SPIROCHETA PALLIDA: Staining of, Zingale, M. Policlinico, Rome **39**: 1435, 1932.

The following method is stated to distinguish spirochetes from pseudospirochetes:

Reagents.—

I.	Saturated alcoholic solution gentian violet	10 c.c.
	Filtered anilin water	100 c.c.
II.	Neutral potassium carbonate	10 gm.
	Distilled water	200 c.c.

Method.—

Place an arbitrary amount (10 to 12 c.c.) of Solution II in a watch glass and add, for each centimeter used 1 drop of Solution I.

Prepare and fix a smear on a cover glass which is then inverted on the surface of the stain in the watch glass. Steam for 3 minutes and wash in running water.

The staining reaction is as follows:

Cellular detritus: Bright violet on pearl gray background.

Bacilli, cocci, and spirilla: deep purple.

Red blood cells: bright lilac.

Spirocheta pallida: deep lilac like borders of erythrocytes.

SPINAL FLUID: Colloidal Benzoin Test, Evans, N., and Dodson, W. R. Am. J. Clin. Path. **2**: 463, 1932.

From a study of 2000 reactions in 1800 cases the authors conclude that the evidence in the literature indicates that of the colloidal tests used in examination of the spinal fluid the benzoin test is the simplest to prepare and read, is as sensitive as and more informative than the gum mastic test, and is more sensitive than and as informative as the colloidal gold test.

A study of the data obtained from some 2000 colloidal benzoin tests on cerebrospinal fluids from 1800 patients signifies that: (a) the benzoin test is not a specific test in the same sense as the Wassermann reaction, (b) it is of value in differentiating active general paresis from other forms of neurosyphilis, (c) it is probably of value in differentiating epidemic encephalitis from poliomyelitis and tuberculous meningitis, (d) a high percentage of positive readings is obtained in disease of the central nervous system, but a positive second zone reading is occasionally obtained in certain conditions not associated with organic disease of the central nervous system.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Recent Advances in Bacteriology*

THE recent advances series published by Blakiston constitutes a most laudable set of volumes. Covering as it does the entire field of medicine and surgery, the volumes together constitute is a very handy reference encylopedia.

So much new has evolved within recent years in pure bacteriology and in clinical bacteriology that the man whose primary interest is not in this field finds it difficult to keep up with the advances or to find readily available within one volume in the literature a summary of the recent clinical advances that have been made. This volume fills that requirement.

Subjects discussed include such as the newer aspects of the streptococcus problem, bacterial variation, rough and smooth types, bacteriophage, Calmette's B.C.G., ultramicroscopic viruses, the specific soluble substances of the pneumococci, haptene antigen, Besredka's local immunity, and the recent work on the anaerobes.

The volume should be of particular interest to clinicians who really want to keep abreast of what is happening in the field of bacteriology and epidemiology.

Recent Advances in Biochemistry**

THIS volume, another of the same series, is especially valuable since it does not start from scratch and saves all of its space for what are truly recent advances, thereby presupposing that the reader is well grounded in his fundamentals to start with. Subjects discussed include structure of proteins and proteolytic enzymes, amino acids, sulphur compounds and protein metabolism, the biochemistry of the fats, carbohydrates, vitamins, sterols, hemoglobin, and the chemical basis of specific immunological reactions. While the entire volume is given over actually to the discussion of the recent advances, the presentation is readily intelligible to one whose primary interest is not biochemistry.

Recent Advances in Medicine†

THIS volume, attempting as it does to cover an extremely wide field, necessarily does so with some sacrifice of detail. The subjects are all well discussed, but with many of them one cannot but regret that the author did not have more space to give them. It serves very well however as a general reference volume for the internist. In specific specialties other volumes in the series may be referred to.

---

*Recent Advances in Bacteriology. And the Study of the Infections. By J. Henry Dible, M.B. (Glas.), F.R.C.P. Professor of Pathology in the University of Liverpool; Late Professor of Pathology in the University of London, and Professor of Pathology and Bacteriology in the Welsh National School of Medicine. Second Edition. With 29 Illustrations. Pages 476. P. Blakiston's Son & Co., Inc., Philadelphia, 1932.

**Recent Advances in Biochemistry. By John Pryde, B.Sc. (St. And.), M.Sc. (Wales) Lecturer in Physiological Chemistry, Welsh National School of Medicine, University of Wales; Formerly 1851 Exhibitioner in Chemistry. Third Edition. With 42 Illustrations. Cloth. Pages 393. P. Blakiston's Son & Co., Inc., Philadelphia, 1931.

†Recent Advances in Medicine. Clinical Laboratory Therapeutic. By G. E. Beaumont, M.A., D.M. (Oxon.), F.R.C.P., D.P.H. (Lond.) Physician, with charge of Out-patients, Middlesex Hospital; Physician to the Hospital for Consumption and Diseases of the Chest, Brompton; Medical Tutor, Middlesex Hospital Medical School; Sometime Radcliffe Travelling Fellow, University of Oxford; and E. C. Dodds, M.V.O., M.D., Ph.D., B.Sc., Courtauld Professor of Biochemistry in the University of London; Director of Courtauld Institute of Biochemistry, Middlesex Hospital; Pathologist to the Royal National Orthopaedic Hospital. Sixth Edition. With 51 Illustrations. Cloth. Pages 442. P. Blakiston's Son & Co., Inc., Philadelphia, 1931.

Recent Advances in Allergy*

THIS is a case in point. While the volume on Recent Advances in Medicine devotes a few pages to Allergy, Bray's volume covers the clinical aspects of Allergy most comprehensively. It is written by a man who himself has made very laudable contributions to the field, particularly in his study of heredity and predisposition in Allergy, and the volume must be classed as paramount with the other recent works on Allergy by other authors.

Recent Advances in Microscopy**

THIS volume is a series of monographs on the biological applications of microscopy as they are seen in medicine, the living eye, zoology, and botany. In the sections on medicine, botany and zoology, most of the discussion is given to the microscopy of the individual cell and this therefore provides a very good compendium of microscopic comparative anatomy. There is much on cellular reproduction. The section dealing with the eye is of particular clinical interest to ophthalmologists since it discusses the principles and the technic of the use of the recently developed Slit-lamp.

Recent Advances in Pulmonary Tuberculosis†

THIS is an especially adequate discussion in a relatively small volume. Subjects discussed include preventive vaccination, x-ray diagnosis (an abundance of good illustrations), sanitarium treatment, sanocrysin, pneumothorax, oleothorax, and the newer surgical methods of collapse.

Recent Advances in Chemotherapy‡

CHEMOTHERAPY, conceived of by Paracelsus, is a science which has been developed by one man, Paul Ehrlich. Ehrlich's original conception based on his side chain theory was that certain chemicals might be hooked on to parasitic organisms thereby destroying their activity without being hooked upon the cells of the host. In the succeeding years this concept has had to be altered since it has been found that with the exception of emetin, certain anthelminthics and possibly Bayer 205, the action is not directly upon the parasite but is indirect through the reaction of the chemical with the body tissues.

The mechanism of parasiticidal activity appears to occur in either of four ways: (1) chemical interaction between the compound as administered or as transformed within the body, and some protoplasmic constituent of the parasite resulting in the death of the latter, (2) physicochemical interaction with the protoplasmic colloid of the parasites resulting in precipitation, coagulation or electrical changes sufficient to destroy or injure the parasite, (3) the production of new compounds within the tissues capable of chemical or physicochemical interaction with the protoplasmic colloid and (4) the production of antibodies within the body due to the release of antigenic substances from the parasites.

---

*Recent Advances in Allergy. (Asthma, Hay Fever, Eczema, Migraine, Etc.) by George W. Bray, M.B., Ch.M. (Sydney) Asthma Research Scholar, The Hospital for Sick Children, Great Ormond Street, London. With Foreword by Arthur F. Hurst, M.A., M.D. (Oxon.), F.R.C.P., Senior Physician Guy's Hospital; Chairman Medical Advisory Committee, Asthma Research Council of Great Britain. 98 Illustrations including 4 Coloured Plates. Cloth. Pages 432. P. Blakiston's Son & Co., Inc., Philadelphia, 1931.

**Recent Advances in Microscopy. Biological Applications. Edited by A. Piney, M.D., M.R.C.P., Director of the Pathological Department, Cancer Hospital, London. Medicine. By A. Piney, M.D., M.R.C.P. The Living Eye. By Basil Graves, M.C., M.A., M.R.C.S., D.O.M.S. (Eng.). Zoology. By E. W. MacBride, Ll.D., D.Sc., F.R.S., and H. R. Hewer, A.R.C.S., D.I.C., M.Sc. Botany. By E. C. Barton-Wright, M.Sc. With 83 Illustrations. Cloth. Pages 260. P. Blakiston's Son & Co., Inc., Philadelphia, 1931.

†Recent Advances in Pulmonary Tuberculosis. By L. S. T. Burrell, M.A., M.D. (Cantab.), F.R.C.P. (Lond.), Senior Physician to Royal Free Hospital; Physician to Brompton Hospital for Consumption and Diseases of the Chest; Consulting Physician to King Edward VII. Sanatorium, Midhurst. Second Edition. With 32 Plates and 17 Text-Figures. P. Blakiston's Son & Co., Inc., Philadelphia, 1931.

‡Recent Advances in Chemotherapy. By G. M. Findlay, O.B.E., M.D., D.Sc., Wellcome Bureau of Scientific Research, London. With a foreword by C. M. Wenyon, C.M.G., C.B.E., M.B., B.Sc., F.R.S., Director-in-chief of the Wellcome Bureau of Scientific Research. London. With 4 Plates and 11 Text Figures. Cloth. Pages 532. P. Blakiston's Son & Co., Inc., Philadelphia, 1930.

This volume reviews the development of our knowledge of specific chemotherapy and the changes in our interpretation of the process. It summarizes very concretely our knowledge of the action of the synthetic chemotherapeutic agents which are in use and many which are still in the experimental stage. It is surprising to observe how many of Ehrlich's original products which he more or less discarded are coming into favor usually under trade names. This is particularly true of the various amebacides and the hypnotics.

Particular attention is given to the chemotherapy of helminthic infection, protozoan infection and bacterial infection. There is also a chapter on virus diseases and one on cancer.

The value of the volume is not so much as a summary of recent work as it is an authoritative treatise on chemotherapy in general. There is only one other volume on chemotherapy available today, that by Kolmer which deals chiefly with syphilis. The two together make a very complete exposition of the subject.

Injuries and Sport*

FOR a thorough résumé of the injuries incident to sports, as well as the everyday routine, I do not believe a better work could be found.

The author has illustrated his examination, diagnosis, and treatment so efficiently with photographs and diagrams that the most difficult cases seem surprisingly simple. Although some of his methods are rather different from the generally accepted American ideas, as a whole the two are practically the same.

The most valuable material in the book is his common sense, practical use of splint and other forms of support.

Any physician or athletic director will do well to have this work in his collection.

Statistical Methods for Research Workers**

THAT this volume, No. V of a series of Biological Monographs and Manuals edited by F. A. E. Crew and D. Ward Cutler, has reached its fourth edition is ample indication of its usefulness to those to whom it is addressed.

The present edition is enlarged by the addition of several new sections and may be accepted as an authoritative presentation of the subject.

This book has been previously reviewed in this JOURNAL and it need only be said that the standard of previous editions is upheld by the present volume.

Laboratory Manual of Physiological Chemistry†

AS implied by the title, this manual is not intended as a reference book, but is designed for the classroom.

It consists of an orderly presentation of well selected procedures designed to constitute a review of qualitative tests relating to physiological chemistry, and illustrative of the qualitative and quantitative procedures available for the analysis of biological material.

Of necessity the choice of methods has been to a definite extent influenced by their didactic value as well as the relative simplicity of the apparatus required.

The fact that the manual has passed into its second edition after a thorough practical trial in the teaching of students both at Johns Hopkins and the University of Pennsylvania is an indication of its usefulness.

For teaching purposes the volume may be recommended as a clear cut, concise, and eminently practical manual.

---

*Injuries and Sport. A General Guide for the Practitioner. By C. B. Heald, C.B.E., M.A. Oxford University Press, American Branch, New York, 1931.

**Statistical Methods for Research Workers. By R. A. Fischer, Sc.D., Chief Statistician, Rothamsted Experimental Station. Ed. 4, cloth, 307 pages. Oliver & Boyd, Edinburgh.

†Laboratory Manual of Physiological Chemistry. By D. Wright Wilson, Benjamin Rush Professor of Physiological Chemistry, University of Pennsylvania. Ed. 2, cloth, 284 pages. Williams & Wilkins Co.

The Journal of Laboratory and Clinical Medicine

VOL. XVIII ST. LOUIS, MO., JANUARY, 1933 No. 4

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - - - - - - - - - CINCINNATI
J. J. R. MACLEOD, M.B. - - - - - - - ABERDEEN, SCOTLAND
W. C. MACCARTY, M.D. - - - - - - - - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - - - - - - - - COLORADO SPRINGS
VICTOR C. MYERS, PH.D. - - - - - - - - - CLEVELAND
RUSSELL L. HADEN, M.D. - - - - - - - - - CLEVELAND
JOHN A. KOLMER, M.D. - - - - - - - - - PHILADELPHIA
ROBERT A. KILDUFFE, M.D. - - - - - - ATLANTIC CITY, N. J.
GEORGE HERRMANN, M.D. - - - - - - - - - GALVESTON
T. B. MAGATH, M.D. - - - - - - - - - ROCHESTER, MINN.
DEAN LEWIS, M.D. - - - - - - - - - - - BALTIMORE
M. H. SOULE, SC.D. - - - - - - - - - ANN ARBOR, MICH.

Contents of this Journal Copyright, 1933, by The C. V. Mosby Company—All Rights Reserved. Entered at the Post Office at St. Louis, Mo., as Second-Class Matter. Additional entry as Second-Class Matter at Fulton, Mo.

EDITORIAL

Paul Gerhardt Woolley

ON THE title page of Volume I, number 1 of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE which appeared in October, 1915, we find the name of Paul G. Woolley, editor for The Department of Physiological Pathology. During the years of his active interest in medicine and medical research, Dr. Woolley was one of THE JOURNAL'S most enthusiastic supporters and few numbers appeared without editorial contributions from his pen. Following the war Dr. Woolley found it necessary to take time off to combat an activated tuberculous infection to which he eventually succumbed at the Desert Sanatorium in Tucson on December 5, 1932.

Paul Gerhardt Woolley was born in Paris, Illinois, April 7, 1875. He received his academic degree from The University of Chicago in 1896 and his M.D. from Johns Hopkins University Medical School in 1900. After serving as Resident House Officer at Johns Hopkins Hospital and Fellow in Pathology at McGill he became Bacteriologist, later Pathologist, and then Director of the Serum Laboratory of the Bureau of Science at Manila. From the Philippines he went to Siam

where he became Director of the Siamese Government Serum Laboratory in 1906 and Chief Inspector of Health in Bangkok, in 1907.

Returning to this country Dr. Woolley accepted the chair of Pathologic Anatomy in The University of Nebraska but after a year he moved to Cincinnati where he became Professor of Pathology in The University of Cincinnati in 1909. For several years thereafter he was Dean of The Faculty of Medicine in The University of Cincinnati Medical School. He was also Director of Laboratories and Pathologist to the Cincinnati General Hospital.

While serving in Siam, Dr. Woolley represented the Siamese Government at the International Congress of Tuberculosis in 1908 and at the International Congress on Hygiene and Demography in 1912. Dr. Woolley lived for a time after the war in Detroit and in Los Angeles but found it necessary to give up his active work.

During the war he held successive ranks from Captain through Lieutenant-Colonel in the Medical Corps and served as Instructor in Military Hygiene at Camp Greenleaf, Epidemiologist at Camps Greene and Devens, and Assistant Camp Surgeon and Sanitary Inspector at Camp Devens.

Dr. Woolley was the author of a volume, "The Clinical History in Outline" (1914), and of a number of papers dealing with subjects of pathology, bacteriology, tropical medicine, and animal parasitology. He served as Associate Editor of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE from its founding to his death. A host of friends join the editor and publishers in regretting the passing of a good fighter.

—*W. T. V.*

CORRESPONDENCE

To the Editor:

In the article by Dr. B. S. Levine in the October number, dealing with sensitizers other than cholesterol in Wassermann and flocculation antigens, he uses the term *sitosterol* to describe the sterol used in the flocculation technic recommended by me in a previous paper (JOURNAL OF LABORATORY AND CLINICAL MEDICINE, **17**: 787, 1932). Strictly speaking, he is correct in that usage insofar as sitosterol is synonymous with phytosterol, the generic term covering all sterols of plant origin. However, some confusion may arise from the fact that the Difco Laboratories sell a specific sterol derived from wheat germ as sitosterol; while the corn germ sterol used in my flocculation antigen has entirely different sensitizing properties.

I originally recommended the wheat germ sterol (Bacto-sitosterol) for use as an adjunct to cholesterol in a Wassermann antigen (*Journal Exper. Med.*, **53**: 605, 1932). I have since found that the flocculation antigen, containing cholesterol and corn germ sterol, is equally serviceable as a Wassermann antigen, and have therefore abandoned the use of wheat germ sterol as an unnecessary complication. Contrary to the findings of Dr. Levine, I find that maximal sensitization with sterols significantly increases the sensitivity of both tests, and in the case of the flocculation test adds greatly to the simplicity of the technic and clarity of the results.

(Signed) HARRY EAGLE

Erratum

In Dr. Lepeschkin's article in the September, 1932, issue of the Journal, page 1251, under *distilled water*, in the second line of the tabular matter, 398.5 c.c. should read 308.5 c.c.

ITEM

The American Association for the Study of Goiter, for the fourth time, offers Three Hundred Dollars ($300.00) as a first award, and two honorable mentions for the best three essays based upon original research work on any phase of goiter presented at their annual meeting in Memphis, Tenn., May 15, 16, and 17, 1933. It is hoped that this will stimulate valuable research work, especially in regard to the basic cause of goiter.

Competing manuscripts must be in English and submitted to the Corresponding Secretary, J. R. Yung, M.D., 670 Cherry St., Terre Haute, Indiana, U. S. A., not later than April 1, 1933. Manuscripts arriving after this date will be held for the next year or returned at the author's request.

The First Award of the Hamilton, Ontario, Canada, 1932 meeting was given Donald McEachern, M.D., Johns Hopkins Hospital, Baltimore, Md., "A Consideration of the Mechanism of Hyperthyroidism Based Upon Its Effect Upon Cardiac and Skeletal Muscle."

Honorable mentions were awarded A. B. Gutman, M.D., Presbyterian Hospital, New York City, "The Effect of Administration of Iodine on the Total Iodine, Inorganic Iodine and Thyroxine Content of the Pathological Thyroid Gland"; Lieut. Col. H. Stott, M.R.C.P., I.M.S., Dean Faculty of Medicine, Lucknow University, Lucknow, India, "The Distribution and Cause of Endemic Goitre in the United Provinces."

Fraternally,
(Signed) J. R. YUNG, M.D.
Corres. Secy.